THE ACTION OF CERTAIN ESTERS AND ETHERS OF CHOLINE AND THEIR RELATION TO MUSCARINE

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INTRODUCTORY

The investigation with which this paper deals started with the observation, that certain specimens of ergot and its extracts exhibited a type of action which was clearly not referable to any of the active principles hitherto described. The action in question appeared to be of the muscarine-type, and, indeed, the search for the principle responsible for it was begun in the expectation that the muscarine would be found. The chemical procedure leading to the isolation of this principle has already been the subject of a paper by A. J. Ewins,[1] who has described its identification as Acetyl-Choline, the intense depressor activity of which was already known through the work of Hunt and Taveau.[2] A preliminary note on the general features of its action has also been published by myself,[3] and it is here only necessary to fill in the details of that account.

For chemical and physiological comparison a specimen of the so-called "synthetic muscarine" or "pseudomuscarine" of Schmiedeberg and Harnack[4] had been prepared, and the opportunity was taken of investigating this substance, with the result that, as elsewhere published, it was proved to be the choline-

[1] Ewins: Biochem. Journ. viii, 44, 1914.

[2] Hunt and Taveau: Brit. Med. Journ., 1906, ii, 1788. Hygienic Lab. Bulletins No. 73, 1911. Journ. Pharm. and Exp. Therap., i, 303, 1909.

[3] Dale: Proc. Phys. Soc. iii, Journ. of Physiol. xlviii, 1914.

[4] Schmiedeberg and Harnack: Arch. f. exp. Path. u. Pharm., vi, 101, 1877.

ester of nitrous acid, $HO \cdot N(CH_3)_3 \cdot CH_2CH_2 \cdot O \cdot NO$ and not, as supposed by Schmiedeberg and Harnack, an aldehyde.

This observation led us to consider the possibility that the true, natural muscarine, from Amanita Muscaria, might be also a choline-ester, although the supposition would involve a revision of the formula, based by Schmiedeberg and Harnack on their analyses. To test this idea we examined several choline-esters, and one of these, the nitric acid ester, $HO \cdot N(CH_3)_3 \cdot CH_2CH_2 \cdot O \cdot NO_2$, was found to resemble true muscarine in action more closely than does the nitrous acid ester—the so-called "pseudo-muscarine." The difference was still clearly marked, however, in that nitric, like the nitrous acid ester, has an action of the nicotine-curare type on the atropinised frog, which is not shown by natural muscarine.

Meanwhile experiments on the stability to hydrolysing agents of the activity of an Amanita extract had considerably weakened the probability of muscarine being a choline ester. Brief boiling with 1 per cent caustic alkali affected the activity of the extract hardly at all. The more stable ethers of choline were, therefore, investigated, and in the ethyl-ether, $HO.N(CH)_3CH_2.CH_2.O.C_2H_5$, we found a substance showing a closer resemblance to muscarine in action than any other of the whole series of derivatives as yet examined from this point of view. The only point of difference was, again, the possession by the ethyl-ether of a nicotine-curare action on the atropinised frog; but this was quite sufficient to exclude its identification with muscarine.

It seems doubtful at present whether we shall succeed in getting further light on the structure of the true muscarine, unless we can procure a sufficient supply of the substance for a direct investigation. Meanwhile it seems desirable to give a comparative account of the action of the chlorine-derivatives which our search has led us to examine, many of them being substances of extraordinary potency, related very closely in their physiological properties, and, in al probability, not distantly in their chemical structure to muscarine itself.

Of the substances examined, only a few, and those the more active, were made the subject of at all a complete physiological

investigation. These were three esters, those of acetic, nitrous, and nitric acids, and one ether, the ethyl-ether. This paper is mainly concerned with the details of the action of these four substances. Concerning numerous less active substances, all bearing some chemical relation to choline, which were tested in the course of the investigation, I can in most cases record only a rough estimate of relative activity, usually based on their effect of the cat's blood pressure.

A few of the experiments have been made on intact animals, with hypodermic injections of the drug. In all other experiments, in which a living animal has been used, it has been fully anaesthetised throughout the experiment, usually with chloroform followed by ether, except in cases where the anaesthetic was stopped after the brain had been completely destroyed.

All the substances tested, except for a few which could be obtained pure commercially, have been prepared synthetically for me by Mr. A. J. Ewins who will elsewhere describe the preparation of such as are new.

THE ACTION OF THE CHOLINE ESTERS OF ACETIC, NITROUS AND NITRIC ACIDS, AND OF CHOLINE ETHYL-ETHER

As stated above all these substances have an action conforming to the general muscarine type, one of them, the nitrous acid ester, having been hitherto commonly known as "synthetic muscarine," though its difference in some features of its action from the natural muscarine has been recognized since Böhm's[5] paper in 1885. It will be convenient to take the different organs and systems separately and compare the actions of the four substances on each in turn. It will be clear that three of the group—the nitrous and nitric acid esters and the ethyl-ether—have an action in most respects closely similar, while acetyl-choline occupies a somewhat unique position, due, as will be suggested, to the facts that it is an extraordinarily active but extremely unstable substance.

[5] Böhm: Arch. f. exp. Path. u. Pharm., xix, 187, 1885.

ACTION ON THE CIRCULATORY SYSTEM

The depressor action of acetyl-choline, even in very minute doses, was emphasised by Hunt and Taveau.[6] They attributed this to weakening of the force of the heart-beat, finding that, with the very low range of dosage within which most of their observations were made, an effect on the rate was unusual. With "very large" doses (e.g. 0.04 mgm. in a rabbit) they observed "great slowing" of the rate. My own interpretation of the effects is somewhat different. With an extremely low range of dose (up to 0.001 mgm.) I agree in finding that the heart-rate is little affected. The fall of blood-pressure which such doses produce I regard as a vaso-dilator effect, finding no evidence of a weakening

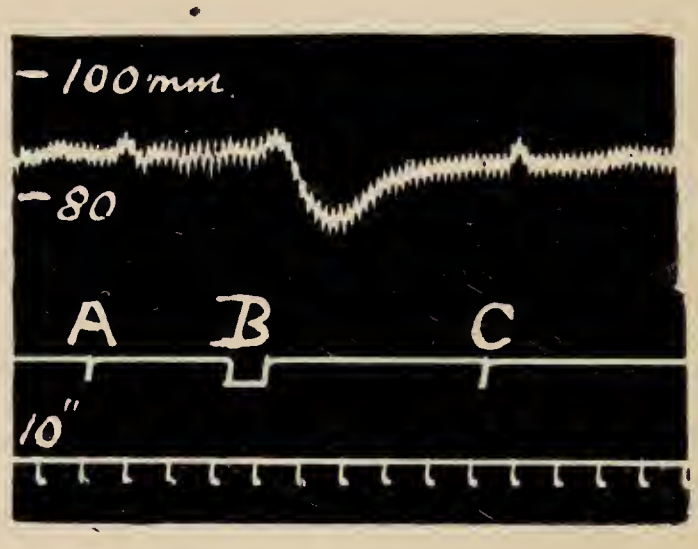


FIG. 1.

Cat: ether. Carotid blood-pressure. At A and C, 2 cc. of normal saline intravenously. At B, 2 cc. of normal saline with 0.000001 mgm. Acetyl-choline.

of the heart-beat. The extent of the fall produced varies with the size of the dose, the anaesthetic used, and the state of tone of the arteries at the moment of injection. The arterial system seems to be more responsive, and a fall therefrom obtainable with lower doses, when the animal is anaesthetised by ether, or by destruction of the brain, than when urethane is used. Even under identical conditions considerable individual variation is observable. The lowest dose with which I have observed a definite fall of pressure, repeated with subsequent similar injec-

[6] Hunt and Taveau: loc. cit.

tions, was the almost incredibly minute amount of 0.000001 mgm. (the one-millionth part of a milligram), which in a cat under ether produced the effect illustrated in figure 1. More commonly the minimal effective dose was of the order of ten times this quantity.

My view as to the vaso-dilator origin of this fall, obtained with minute doses of acetyl-choline, is based on experiments with the myocardiograph (Cushny), with the plethysmograph and with the perfused isolated organ. Figure 2 shows the effect on the

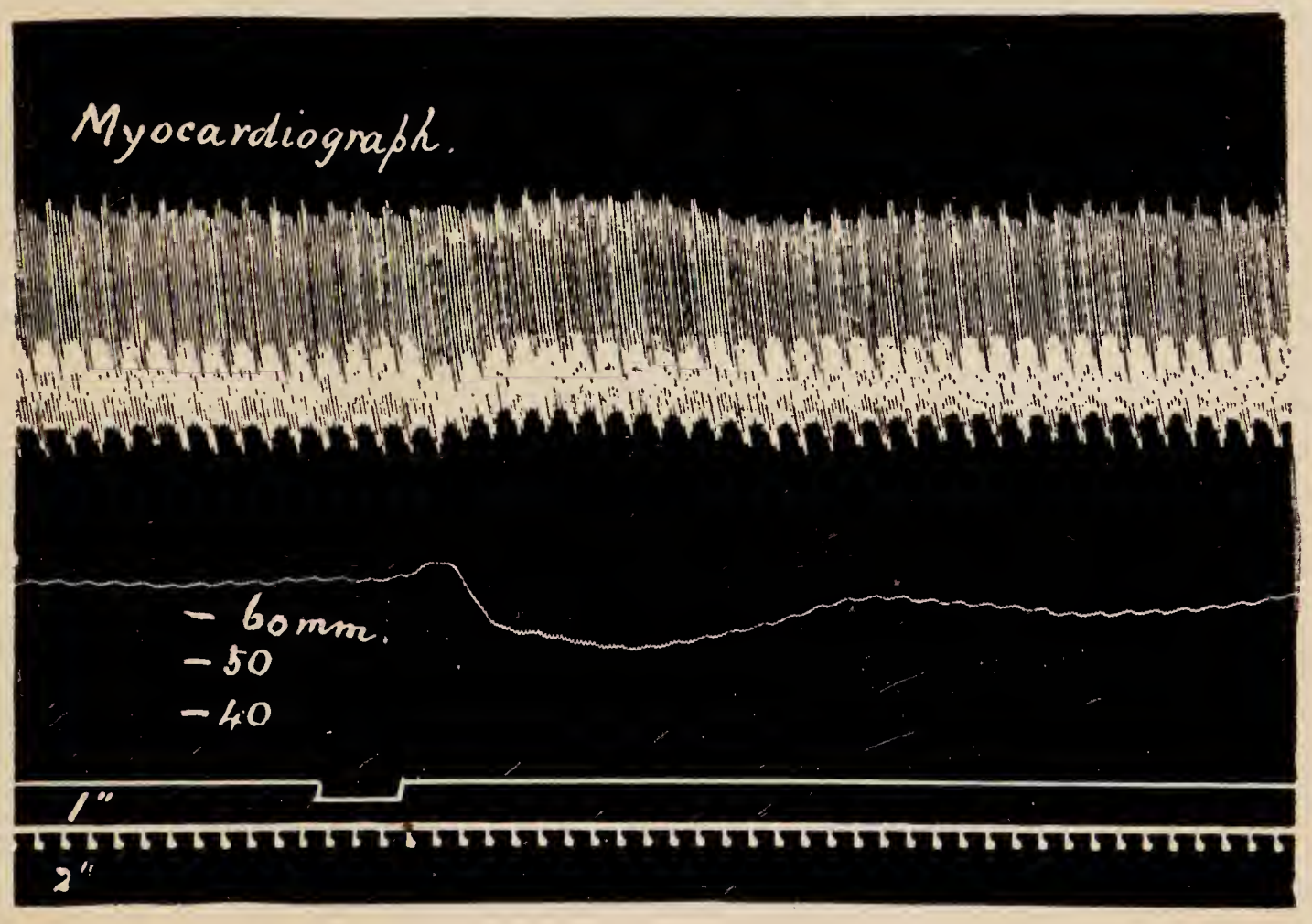


FIG. 2.

Cat: brain destroyed. Ventricular contractions and carotid blood-pressure. Intravenous injection of 0.001 mgm. Acetyl-choline.

blood-pressure and the contractions of the left ventricle in a cat, with brain destroyed. It will be seen that the fall of blood-pressure produced by injecting 0.001 mgm. of acetyl-choline is not accompanied by any measureable change in the amplitude or rate of the ventricular contractions. Figure 3 shows that an increase in the volume of an intestinal loop and of a limb accom-

panies the fall of blood-pressure produced by such a dose. The volume records are from different experiments, and have been superposed for convenience of reproduction; the size of the animals, method of anaesthesia, and the effect on the blood-pressure, were practically identical in the two cases.

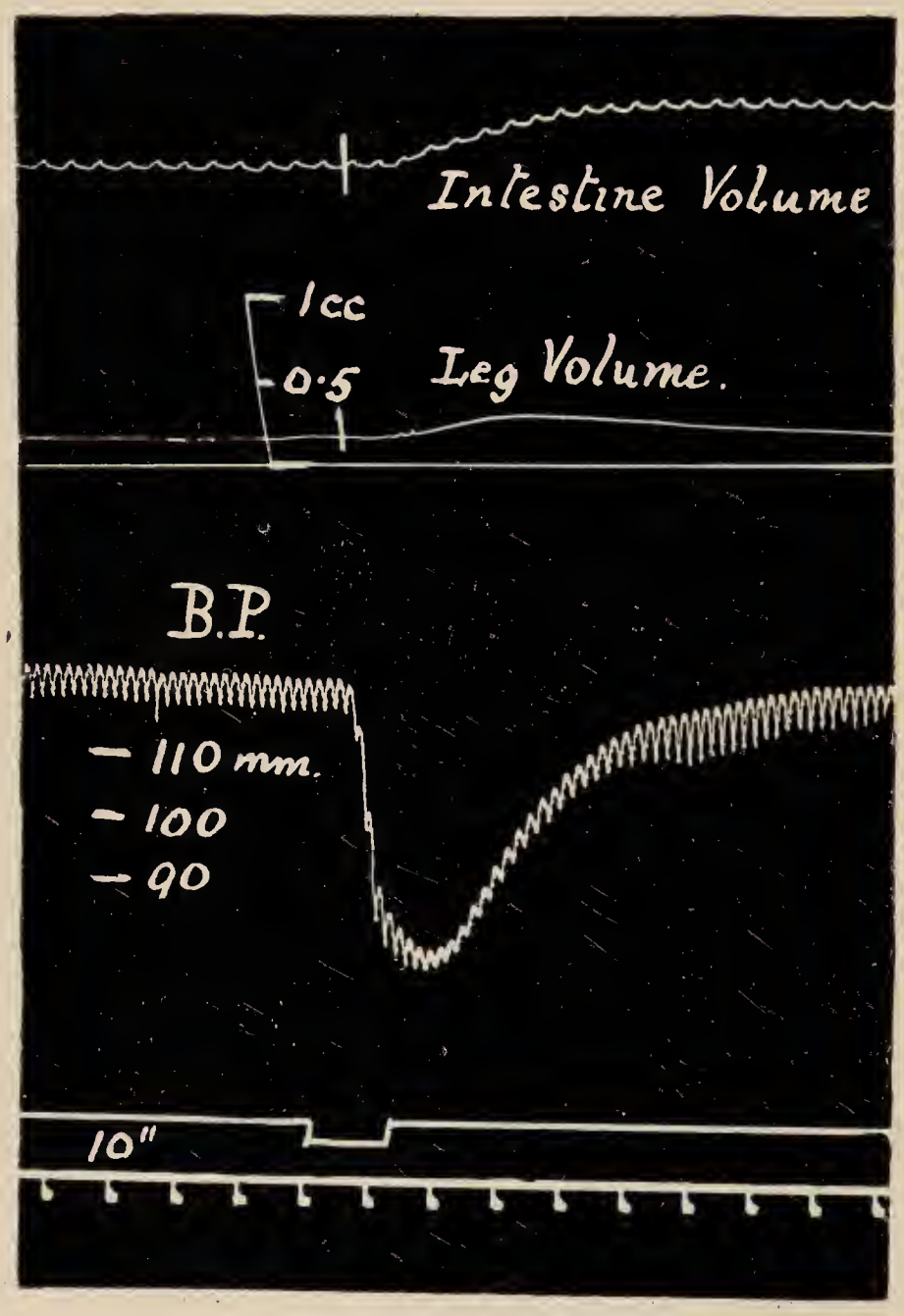


FIG. 3.

Cat: ether. Plethysmograph records from intestine and limb. Carotid blood-pressure. Injection of 0.001 mgm. Acetyl-choline.

Figure 4 demonstrates directly the vaso-dilator action of the ester, being taken from the drop record from a rabbit's ear, perfused with Ringer's solution at the room-temperature, according

to the method of Krawkow and Bissemski,[7] as modified by Rischbieter.[8]

It will be noted that the effects of these purely vaso-dilator doses of acetyl-choline are small. Larger effects of the same type cannot be obtained, however, since if the dose is pushed a little higher another action begins to complicate the record, an intense, though evanescent action on the heart making its appearance, usually when the dose reaches the order 0.01 mgm. The effect bears a remarkable resemblance to that of a brief faradisation of the vagus nerve, but is usually followed by a secondary fall of pressure during which the heart-beat is quickened even beyond its original rate. Typical examples of this sequence of effects—immediate, intense slowing of the heart-rhythm, followed by a period of quickened rhythm, during which the pressure keeps low or even falls further, and then gradually returns to the

FIG. 4.

Upper line = drop-record from perfused rabbit's ear. Lower line = time signal. Effect of injecting 0.005 mgm. Acetyl-choline into the arterial perfusion cannula.

normal—can be studied in figures 5 and 6. When once the normal has been regained, which, even with doses of the order of 1 mgm. always occurs after a few minutes, the whole effect can be reproduced in all its features by giving another, similar injection. The evanescence of the effect, and the regularity of its reproduction with successive doses, which, as we shall see, are characteristic of all the actions of acetyl-choline, may be connected, with some probability, with the readiness with which the ester is hydrolysed into its relatively inert constituents, choline and acetic acid. Ewins and I found that such hydrolysis occurs with great rapidity in watery solutions made distinctly alkaline, even at the

[7] Bissemski: Wratsch, 1912, No. 8.

[8] Rischbieter: Zeitschr. f. d. ges. exp. Med., i, 355, 1913.

ordinary room temperature. Even Ringer's solution containing the ester in high dilutions loses activity at a perceptible rate in the cold. In the blood at body temperature it seems not improbable that an esterase contributes to the removal of the active ester from the circulation, and the restoration of the original condition of sensitiveness. The similarly rapid recovery after a dose of adrenine, which is oxidised in alkaline solution

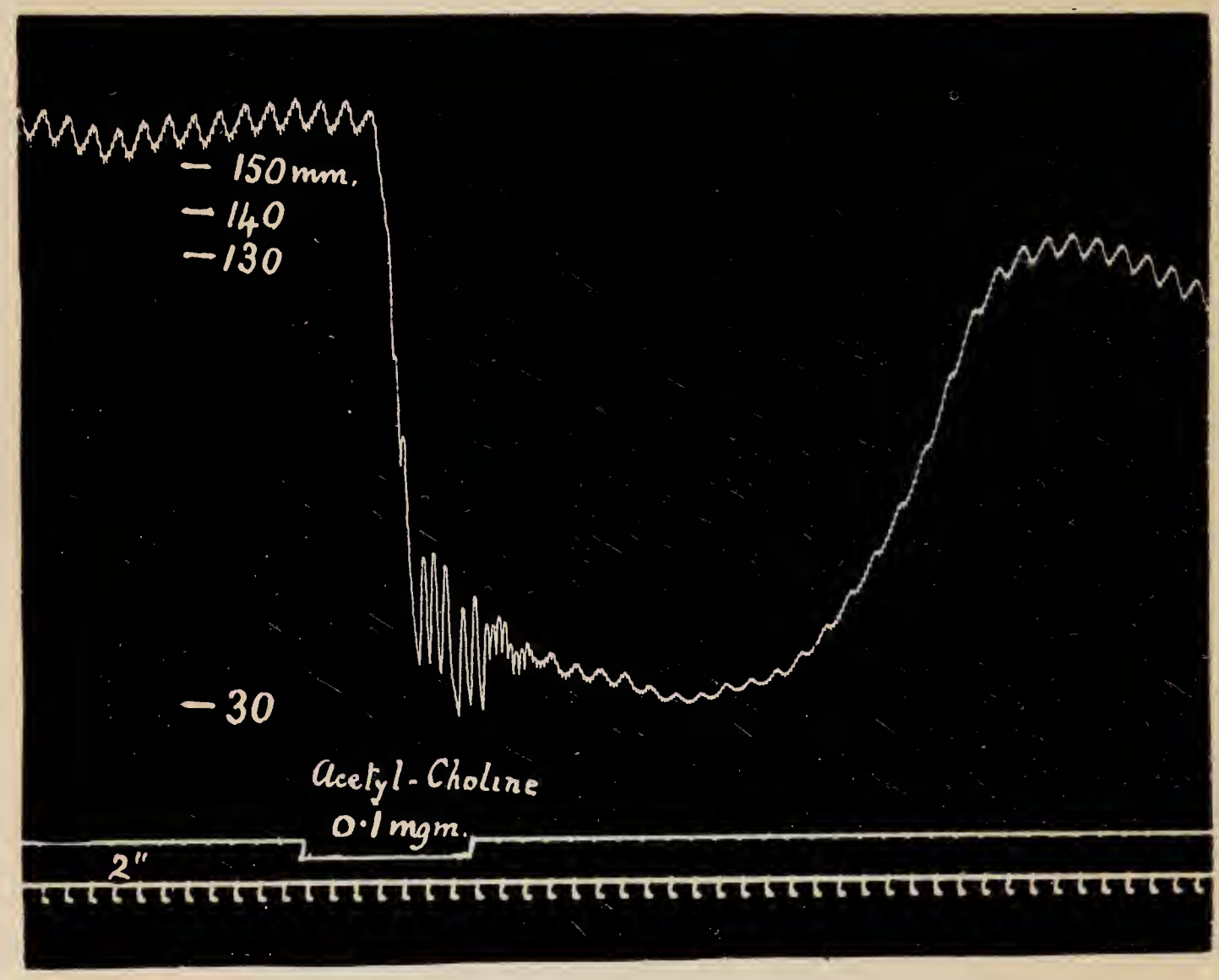


FIG. 5.

Cat: brain destroyed. Artificial respiration. Effect on carotid blood-pressure of injecting 0.1 mgm. Acetyl-choline intravenously.

only less easily than acetyl-choline is hydrolysed, suggests itself as an obvious parallel.

The action of the nitrous acid ester (synthetic muscarine) on the heart and blood-pressure differs from that of acetyl-choline chiefly in being less intense, but more persistent. I have not, in

this case, analysed the effect of small doses; but, since falls of blood-pressure can be obtained with injections of 0.001 to 0.01 mgm. of this ester, which are superficially very similar to those produced by doses of acetyl-choline one hundred times smaller, there is a strong presumption in favour of the effect of such doses being mainly due to vaso-dilatation. The nitroso-choline is, in any case, a far less potent vaso-dilator than a acetyl-choline is

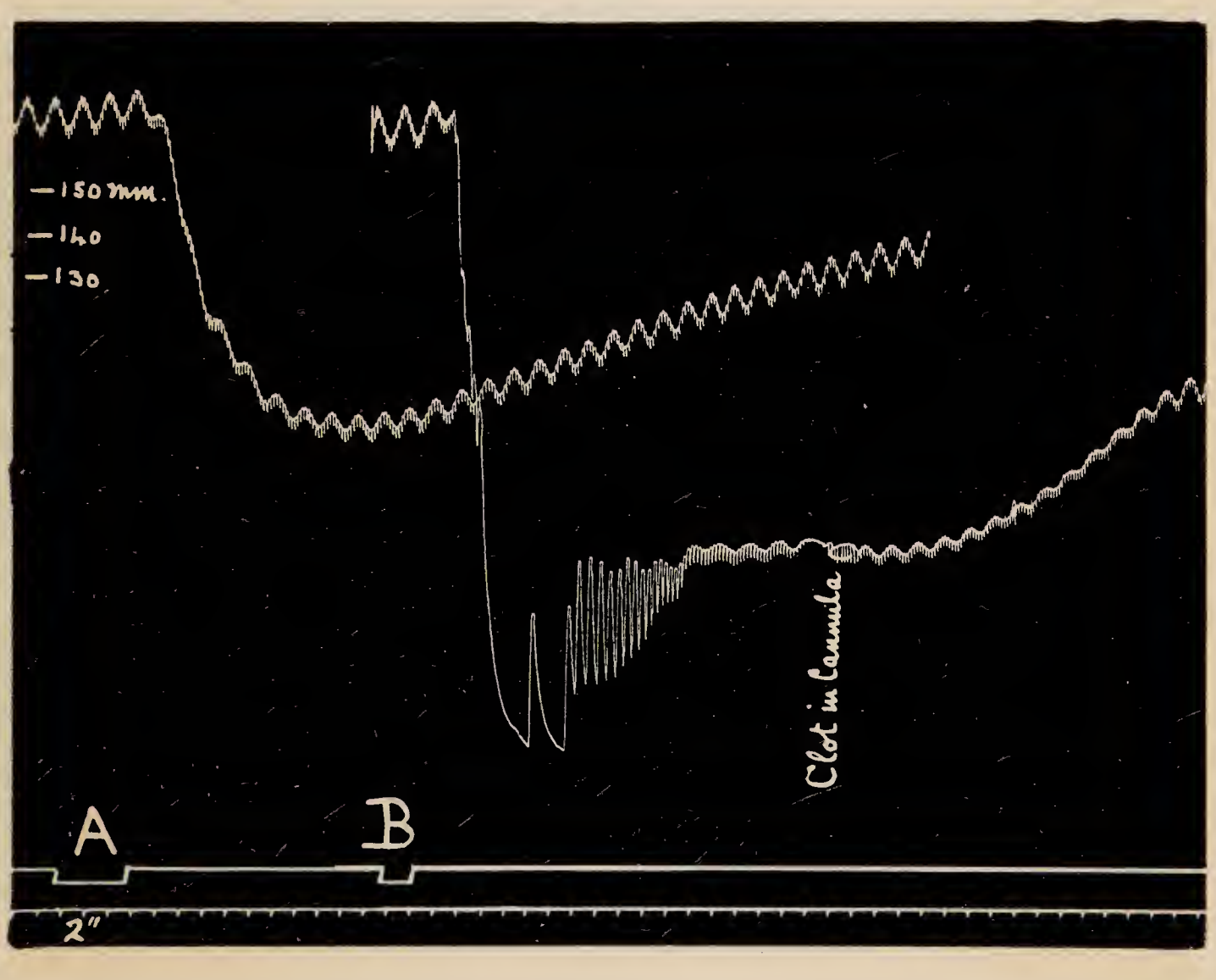


FIG. 6.

Cat: ether. Carotid blood-pressure. Intravenous injections. At A 0.1 mgm. Choline nitrous ester, at B 0.1 mgm. Acetyl-choline.

interesting, in view of the marked vaso-dilator effect of nitrites in general, and of other esters of nitrous acid in particular. As the dose is raised a cardioinhibitor effect makes its appearance, not as an intense initial phase of the action, as in the case of acetyl-choline, but as an effect more moderate in degree, slower in onset, and at the same time much more persistent. The vaso-

dilator effect of smaller doses, if such it be, becomes, in fact, gradually obscured by the cardiac inhibition as the dose is pushed higher, until, with doses of 0.5 mgm. or more, a prolonged slowing of the heart-beat is the main visible effect on the circulatory system, constituting the typical "muscarine" action. Figure 6 shows a comparison between the effects of 0.1 mgm. doses of the acetic and nitrous acid esters, injected in succession into the same cat under ether. It will be seen that this dose of the nitrous ester

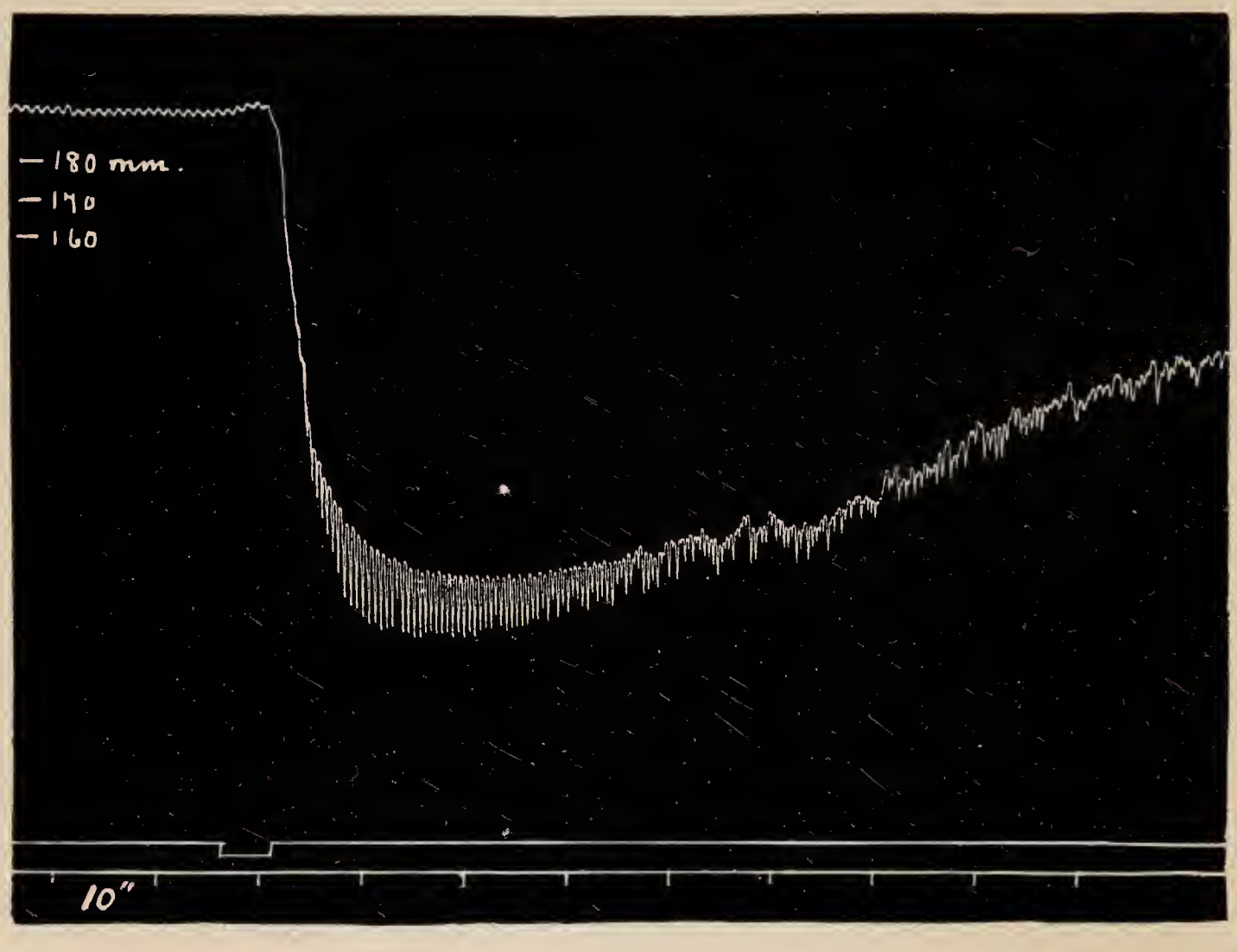


FIG. 7.

Cat: ether. Carotid blood-pressure. Intravenous injection of 1 mgm. Choline nitric ester.

causes a considerable fall of blood-pressure, without any noteworthy inhibition of the heart, while the same dose of acetylcholine causes the characteristic complex of initial vagus-like action of the heart, followed by a rather slower depressor effect, accompanied by some acceleration of the beat.

The nitric acid ester of choline has an action on the circulatory

system which is not distinguishable from that of the nitrous acid ester (fig. 7). The ethyl-ether, again, produces a very closely similar effect, (cf. fig. 17) though its action appears to be somewhat more powerful, and especially more persistent than that of the nitrous and nitric acid esters. The greater persistence, with action of the same order of intensity, might be expected, in view of the greater resistance of the ether to hydrolysis.

Briefly summarising the action of these four substances on the heart and circulation, when they are injected intravenously into the anasthetised mammal, we have in all cases a depressor action with very small, in the case of acetyl-choline with extremely minute doses, which seems to be mainly a vaso-dilator effect. With larger doses all produce marked inhibition of the heart-beat: with acetyl-choline this appears with doses as low as 0.01 mgm., but the other three must be given in doses of the order of 1 mgm. to produce a very intense inhibition. In the case of acetyl-choline the cardiac effect is extremely rapid in onset, intense in degree, and evanescent; the other three substances produce an effect of slower onset, lower maximal intensity, but much greater persistence.

ACTION AFTER NICOTINE AND ATROPINE

These effects on the blood-pressure are purely peripheral in their origin. Neither section of the vagi, nor the injection of nicotine in large doses, diminishes the cardioinhibitor effect. The production of vaso-dilatation in the perfused rabbit's ear has already been described.

Atropine, on the other hand, readily and completely abolishes the depressor action and the cardioinhibitor action, and brings to light an action of another type, seen best in a cat which has had the spinal cord cut in the neck and the brain destroyed under preliminary anaesthesia, and which is then kept under artificial respiration with pure air. If the arterial pressure of such an animal is recorded, and 1 mgm. of atropine sulphate injected intravenously, it is found that all the four choline derivatives have lost their depressor and cadioinhibitor effect completely, but that any

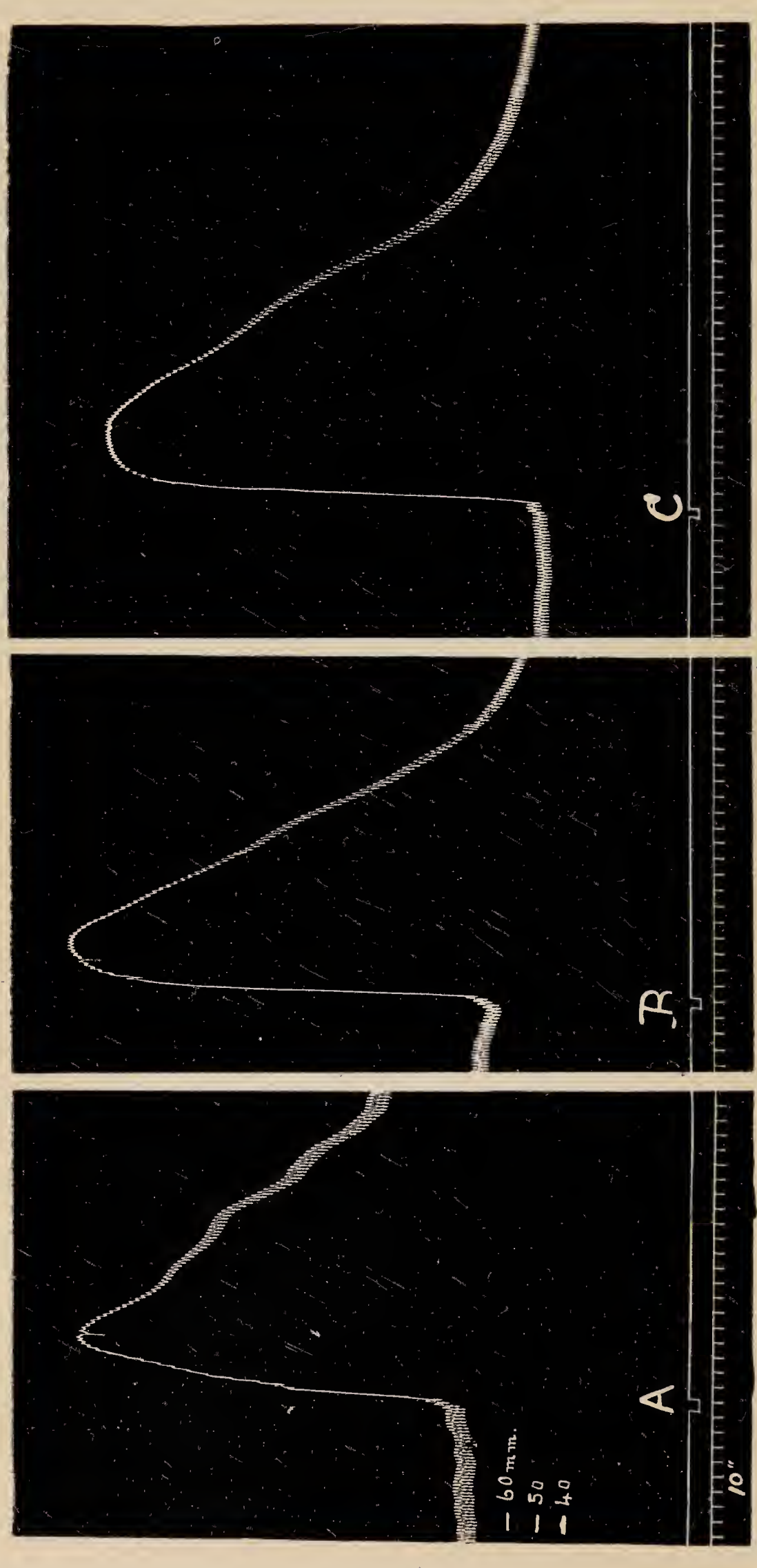


FIG. 8.

Cat: brain destroyed. 1 mgm. Atropine previously injected. Intravenous injections. A, B and C of 5 mgm. each of choline esters of acetic, nitrous, and nitric acids.

of the three esters, given in a large dose, such as 5 mgms., will produce a very large rise of pressure, having a superficial similarity to that produced by 1 mgm. or less of nicotine (fig. 8). The ethyl-ether, on the other hand, has very little effect of this type, which, it may be noted, is also very weak or absent in the case of natural muscarine.

This pressor action after atropine was described by Böhm[9] in the case of "synthetic muscarine" (i.e., the nitrous acid ester). It is not a central effect, for it can be obtained after destruction not only of the bulbar centers, but of the whole spinal cord. On the other hand it is completely abolished by a dose of nicotine

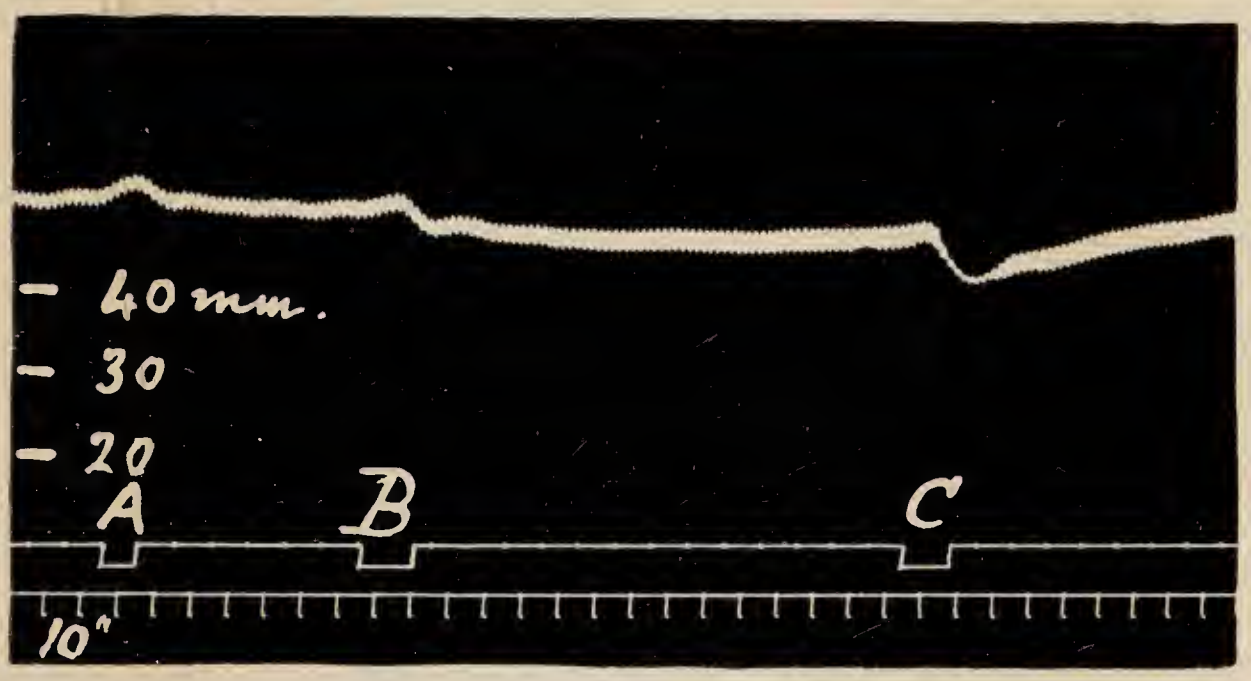


FIG. 9.

From same experiment as Fig. 8, after further injection of 30 mgm. Nicotine tartrate. Intravenous injections, at A 5 mgm. Choline nitrous ester, at B 10 mgm. Choline nitric ester, at C 10 mgm. Choline acetic ester.

sufficient to paralyse all the ganglia of the involuntary nervous system—e. g. 30 mgm. for a cat (fig. 9).

These choline-esters have, then, two quite distinct types of action on the heart and circulation—the depressor, cardioinhibitor "muscarine" type of action, unaltered by nicotine, but abolished by atropine, and a pressor action of the nicotine type, unaffected by atropine, but abolished by large doses of nicotine.

[9] Böhm: loc. cit.

This secondary type of action is, of course, only seen when the primary, muscarine-like action, has been abolished by atropine. The secondary action being obviously of the nicotine-type, it was of interest to enquire whether increased output of adrenine from the suprarenal glands played any considerable part in its production. When the suprarenal glands are excised the esters still produce a large rise of arterial pressure after atropine, but this is, apparently, rather less than that which they normally cause. In this respect, again, their action appears to be of the nicotine type.

DUAL ACTION OF CHOLINE ON BLOOD-PRESSURE

The discovery of this marked duality in the action of choline-esters raised the question of its existence in the case of choline itself. Much controversy has arisen over the question whether the action of pure choline is depressor or pressor; Popielski and his coworkers[10] maintaining that perfectly pure choline has a pressor action, the depressant effects obtained by other observers[11] being, in his opinion, due to decomposition products. Choline is such a stable substance, and so easily obtained by synthesis in a state of perfect purity, that it seemed more likely that the discrepancy of observation between the different observers depended on a duality of action, the conditions employed and doses administered being such as to bring out the muscarine-like action in some hands, and to give predominance to the nicotine-like action in others. In that case we must regard esterification as intensifying both types of action. This seems to be the most probable explanation. The specimen of choline which I have used is a very pure synthetic hydrochloride, which, when injected intravenously in doses of 1 to 10 mgm. into a cat under ether, with

[10] Popielski: Zeitsch. f. physiol. Chem., lxx, 250, 1910. Modrakowski: Pflüger's Arch., cxxiv, 601, 1908.

[11] Cf. for example, Mott and Halliburton, Phil. Trans. B., cxci, 211, 1899.

Abderhalden and Müller, Zeitschr. f. physiol. Chem., lxv, 420, 1910, and lxxiv, 253, 1911.

Mendel, Underhill and Renshaw: Journ. of Pharm. and exp. Therap., iii, 649, 1912.

high blood-pressure, produces a pure fall of blood-pressure, very similar to that caused by small doses of the nitrous or nitric ester, or of the ethyl ether. On the other hand, in the pithed cat, which has received a small dose of atropine, larger doses, (e.g. 25 mgm.) of the same sample of choline produce a pure rise of blood-pressure. A similar observation has been made by several of the previous workers on this point. The action, though much weaker, is obviously of the same type as that produced by the esters under the same conditions, since, as in

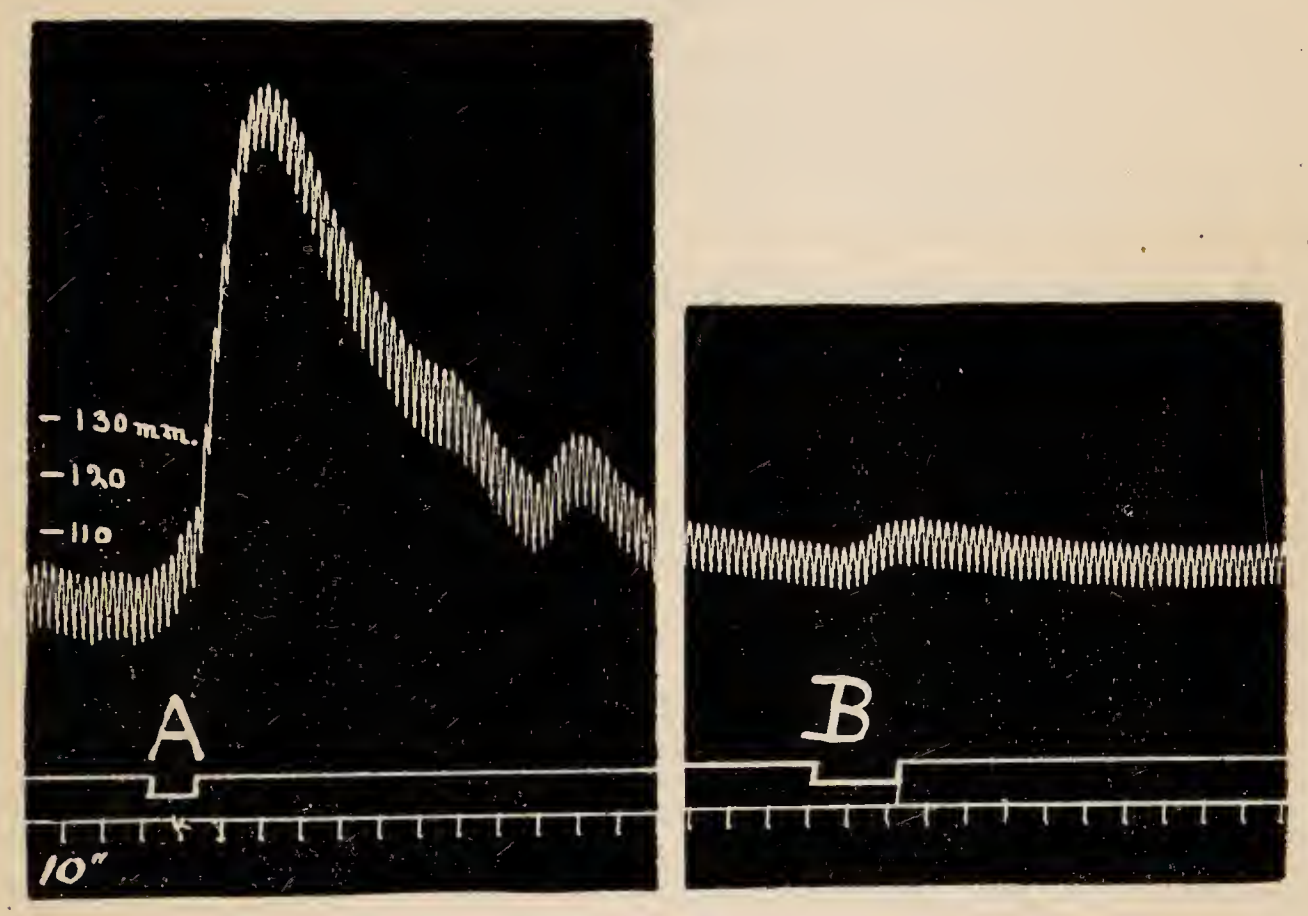


FIG. 10.

Cat: brain destroyed. 1 mgm. Atropine previously. At A 25 mgm. Choline chloride intravenously. At B, after 30 mgm. Nicotine, 50 mgm. Choline chloride.

the latter case, the effect is completely abolished by a paralytic dose of nicotine (fig. 10).

ACTION ON THE ISOLATED HEART

It was suggested that the evanescence of the effect of acetylcholine was due to the ease with which it is hydrolysed into relatively inert constituents. If this is so, it is clear that no accurate

idea of its relative activity, as compared with those of the other derivatives under examination, can be obtained by the method of intravenous injection. The only satisfactory comparison will be that afforded by perfusion of the bases, in known dilutions, through some isolated organ. Even in Ringer's solution, when warmed, acetyl-choline in high dilutions seemed to be hydrolysed with considerable rapidity. The frog's heart was, therefore, chosen for the comparison. In Ringer's solution at room-temperature acetyl-choline disappears but slowly, though even under such conditions the weaker dilutions show a perceptible loss of activity after standing for some hours.

The Ringer's solution mostly used was made up according to a formula recently given by Clark.[12] The perfusion was carried out with the aid of the 5-way cannula described by Mines,[13] which enables an immediate change of solution to be carried out without alteration of the pressure or interruption of the flow. The nozzle was tied either into the vena cava, or into the sinus venosus itself. The apex of the ventricle was attached by a thread to a lever, which it pulled downwards with systole, against the tension of a light spring. A series of records obtained with one heart is shown in figure 11. It will be seen that, under these conditions, acetyl-choline has an action on the frog's heart far beyond that recorded even for natural muscarine, complete stoppage of the ventricle being obtainable with one part of ester in 100 millions of solution, and a perceptible inhibition even with one part in 1000 millions. The effect of the nitrous ester is obviously much weaker, 1 part in 100,000 stopping the heart in a similar manner to 1 part of the acetic ester in 100 millions; while the effects of one part of the nitrous ester in one million and of one part of the acetic ester in 500 millions show a close similarity. Acetyl-choline would appear, therefore, to be about 500 times as strong as the other in action on the frog's heart, when tested under the simple conditions of perfusion. It may be noted in passing that the comparatively low value obtained for the activity of the nitrous ester on the frog's heart is the one apparent dis-

[12] Clark: Journ. of Pharm. and exp. Therap., iv, 403, 1913.
[13] Mines: Journ. of Physiol., xlvi, 190, 1913.

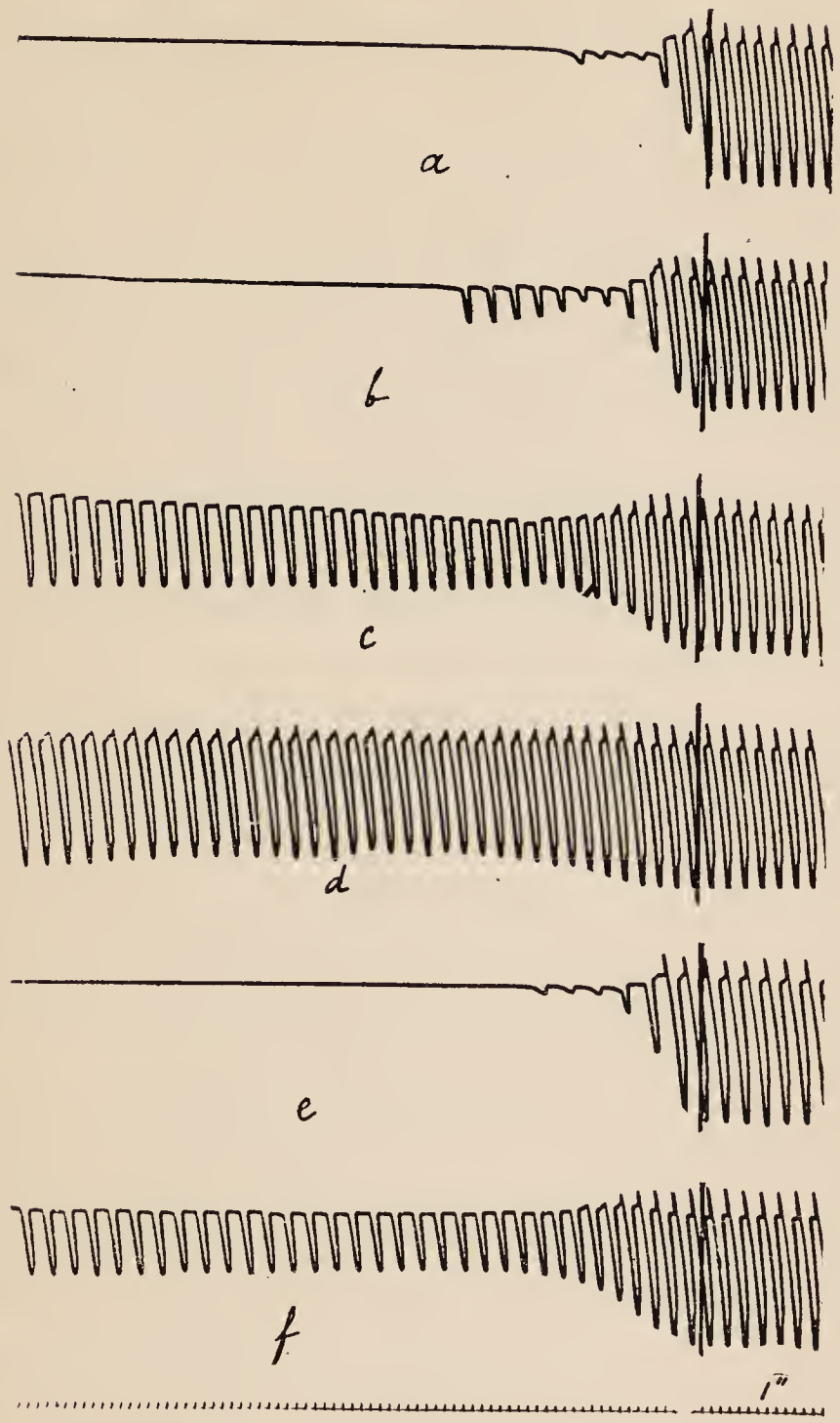


FIG. 11.

Perfused heart of frog, recorded by suspension-lever. Tracings read from right to left, the vertical line in each case indicating change from pure Ringer's solution to similar solution containing a choline-ester.

a Acetyl choline 1 in 100 millions.
b Acetyl choline 1 in 200 millions.
c Acetyl choline 1 in 500 millions.
d Acetyl choline 1 in 1000 millions.
e Nitroso-choline 1 in 100,000.
f Nitroso-choline 1 in 1 million.

crepancy between my observations on this substance and those of previous workers on "synthetic muscarine." In the most recent paper giving quantitative determinations of the activity of the latter, Honda,[14] working with the same species of frogs (R. temporaria) and at the same time of year (February), obtained permanent stoppage of the heart with 1 part in 1 million of perfusion-fluid. This would seem to indicate an activity about 10 times as great as that which I observed. Honda was using Williams' apparatus, in which the heart works against a pressure, which may have affected the result. But the more probable source of the apparent discrepancy is a difference in the perfusion-fluid. In a few experiments which Mr. Mines kindly made for me at Cambridge, in which a Ringer's solution containing sodium borate in place of sodium bicarbonate was used, 1 part of the nitrous ester in 50,000 of Ringer's solution failed to stop the heart; but, since in the same experiments one part of acetylcholine in 250,000 produced only a temporary stoppage for a few beats, it was clear that a small difference of conditions could very seriously change the scale of the effective dosage, and that no significance could be attached to the apparent quantitative discrepancy between Honda's results and my own.

I was unable to detect any difference between the action of the nitric and nitrous esters on the frog's heart perfused in this manner. The ethyl ether, however, showed itself definitely somewhat more active than these two esters, the activity-ratio being about 2:1 and 5:1 at different stages of the same experiment. The ethyl ether was, therefore, much less active than the acetic ester when thus applied.

Like muscarine these choline derivatives exhibit not only quantitative but qualitative differences of activity on different frog's hearts. In one instance a dilution of any of them strong enough to produce an ultimate arrest, will affect the strength of individual beats but little, stoppage being attained by a progressive increase of the diastolic pause to infinite duration. In another case the effect is almost entirely on the strength of the

[14] Honda: Arch. f. exp. Path. u. Pharm., xlv, 454, 1911.

beats, which have become rapidly smaller, with very little widening of the intervals between them, till they become vanishingly small. All types of action intermediate between these two extremes can be observed.

While acetyl-choline is by far the most powerfully active of the four derivatives when thus perfused, it is the weakest of them when the solution is dropped on to the surface of the exposed heart of a pithed frog, with natural circulation. Under such conditions even a 2 per cent solution of acetyl-choline, though producing marked slowing or weakening of the beat, is usually inadequate to cause complete arrest. The nitrous and nitric acid esters may cause complete stoppage of the frog's heart when applied to its surface in 1 per cent solution, but not infrequently fail. Under these conditions the ethyl-ether is by far the most active of the four derivatives, application of a 0.1 per cent solution rarely failing to stop the heart. The order of the activities judged by this test, is quite different, therefore, from that obtained by the perfusion method. Clearly some other factor is involved, and it is reasonable to suppose that this is the hydrolytic action of the blood, which makes acetyl-choline, by far the most active of the four under conditions excluding this complication, appear to be the least active, and gives to the activity of the stable ethyl-ether a fictitiously high relative value.

ACTION ON OTHER INVOLUNTARY MUSCLE

The Alimentary Canal. All the four choline-derivatives are powerful stimulants of the activity of the muscular walls of the whole alimentary canal—oesophagus, stomach, small and large intestines. When they are injected intravenously into the animal under anaesthetic, or hypodermically into the intact animal, acetyl-choline appears to be very much weaker in action on these structures than the other three derivatives. To produce any marked stimulation of intestinal movements it must be given intravenously in relatively large doses—e.g. 0.5 mgm. Even then the effect which it produces is markedly evanescent (fig. 12). The other three produce more powerful, much more prolonged

intestinal contractions in smaller doses than this, such as 0.1 mgm. The effect of such doses on the heart and circulation are, as already shown, small in comparison to those produced by even an

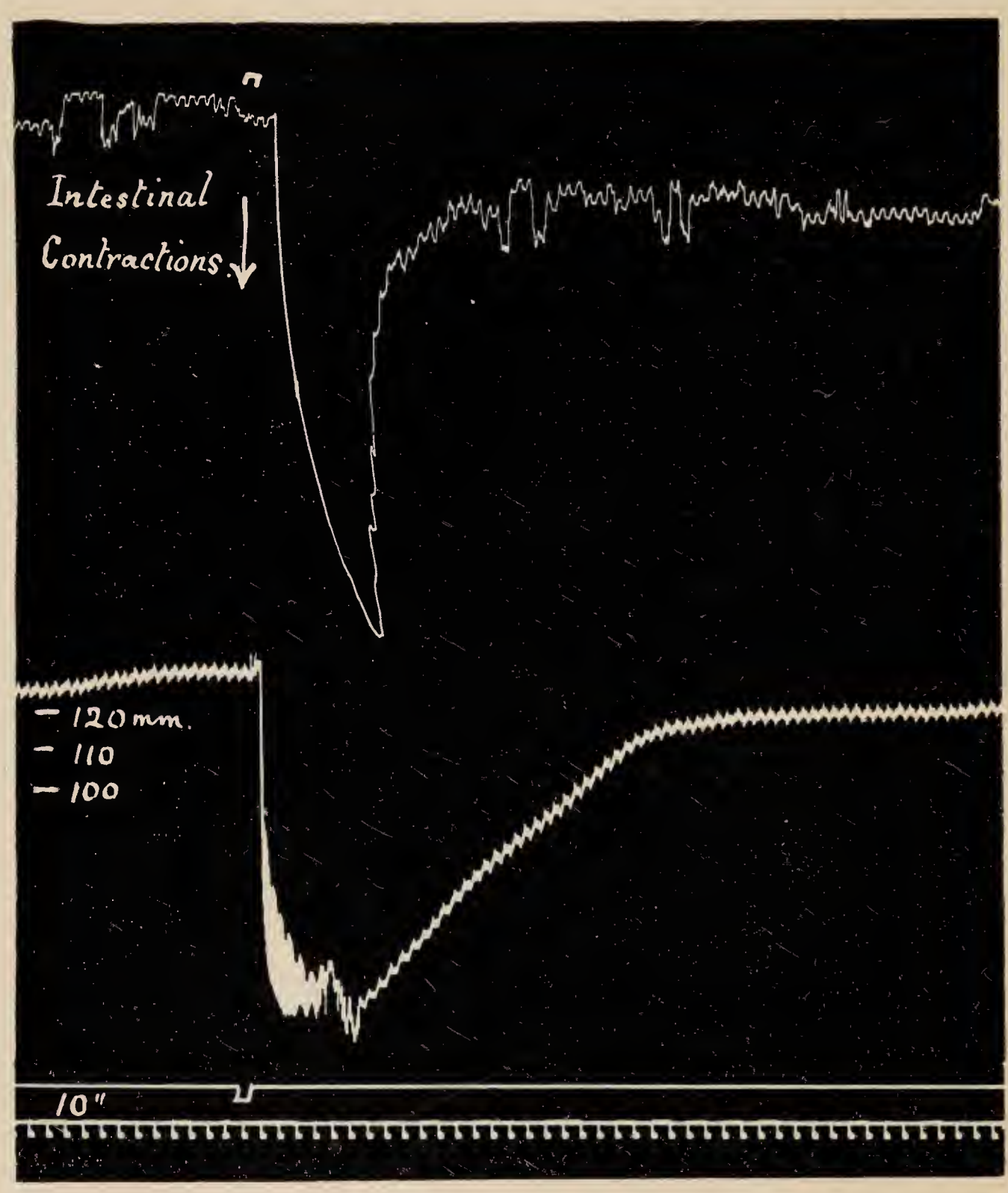


Fig. 12.

Cat: ether. Contractions of small intestine (Trendelenburg's method). Carotid blood-pressure. Intravenous injection of 0.5 mgm. Acetyl-choline.

equal dose of acetyl-choline (Cf. fig. 7). It would appear, at first sight, therefore, that acetyl-choline differs from the other

choline derivatives in being more powerful in its action on the circulatory system, and less powerful in its action on other involuntary muscle, such as that of the alimentary canal. This appearance is, however, illusory. When a loop of intestines is isolated from the body and suspended in Ringer's solution it exhibits a very high degree of sensitiveness to the action of acetyl-choline, as to that of the other esters and the ethyl-ether. In

FIG. 13.

Loop of Rabbit's small intestine in 50 cc. Tyrode's solution. At A 0.01 mgm. synthetic Acetyl-choline, at B 0.01 mgm. Acetyl-choline from ergot, added to the bath. At R, R, fresh Tyrode's solution.

the course of the investigation which led to the isolation of acetyl-choline from ergot, I used as a test-object, for the active principle which we were seeking, a loop of rabbit's small intestine, suspended in warm oxygenated Tyrode's solution. In common

with other observers, I find this a much more suitable medium for the maintenance of regular activity of isolated intestinal muscle than a Ringer's solution made up according to the older and more generally useful formulae, such as that of Locke. Figure 13 is a record in the final stage of the identification of acetyl-choline in ergot, and shows the sensitiveness to this substance of the rabbit's intestinal muscle suspended in Tyrode's solution, the concentration of acetyl-choline in the bath, in the

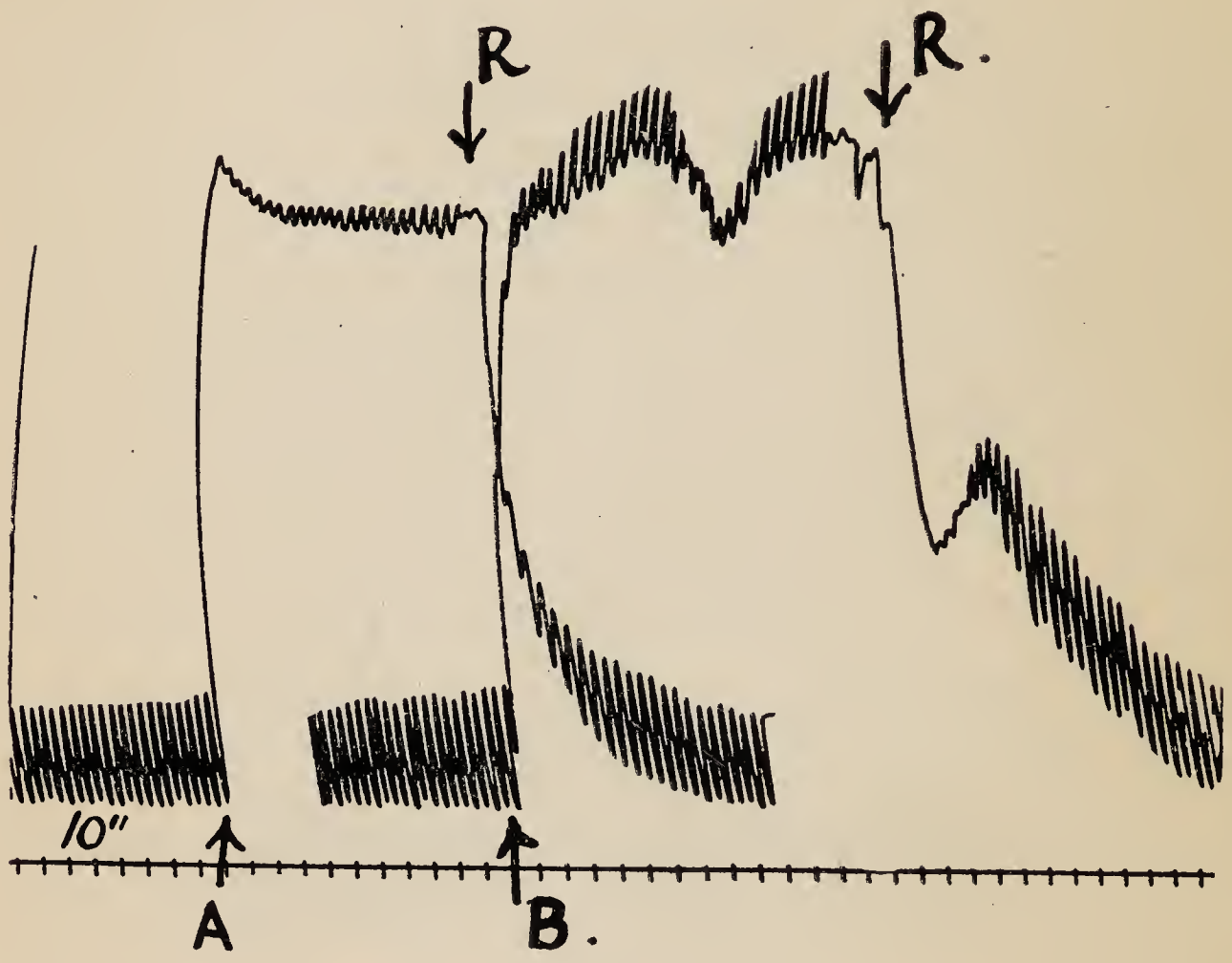


Fig. 14.

Similar to Fig. 13. At A 0.05 mgm. Acetyl-choline, at B 0.05 mgm. Choline nitrous ester.

case of each dose, being 1 in 5 millions. A distinct though not maximal reaction can sometimes be obtained with dilutions of a much higher order than this, a sensitive preparation showing definite augmentation of activity when acetyl-choline to the extent of 1 part in 500 millions is added. The sensitiveness is, therefore, sometimes of the same high order as that of the frog's heart.

The nitrous and nitric esters and the ethyl ether, though all three, when injected into the whole animal, have, as already stated, a much more powerful action on the muscle of the alimentary canal than acetyl-choline, are somewhat less active than the latter on the isolated intestine, though it is difficult to assign a precise numerical value to the difference. When given in moderate doses, so as to make the dilution of base in the bath something between 1 in 5 millions to 1 in 500,000, acetyl-choline produces but little more effect than the other three: indeed with the higher doses it may produce less. Figure 14 shows the effects of the acetic and the nitrous acid esters, each added in the proportion of one part in one million. The effects of the ethyl ether and nitrous and nitric esters are practically indistinguishable, when the three are compared against one another on the same isolated preparation. The effect of acetyl-choline differs from those of the other three derivatives, in moderate doses, rather in its greater rapidity of onset than it its ultimate intensity. With very low dosage acetyl-choline becomes decidedly superior, a preparation, which gives a definite response to one part of this ester in 500 millions, needing 10 times this concentration of any of the other three compounds to produce a similarly distinct effect.

The readiness with which acetyl-choline is hydrolysed in an alkaline medium has already been suggested in explanation of the evanescence of its effect on the blood-pressure. Its comparatively weak effect on the plain muscle of the alimentary canal, when it is given intravenously, when viewed in conjunction with its very intense action on the same type of muscle isolated from the body, confirms the suggestion that its destruction in the body is extremely rapid. It may be pointed out that injection into the circulation brings it with great rapidity into contact with the involuntary muscle of the heart and arteries, so that the minutest doses are capable of producing circulatory effects, though its ready destruction makes all its effects evanescent. The extra delay involved before it reaches the muscular coats of the alimentary canal or other viscera apparently allows a considerable further destruction to take place, so that a wholly false impres-

sion is given of its relative activity on these structures as compared with that of the other more stable choline derivatives.

An interesting contrast may be drawn between the action of these choline derivatives, on the one hand, and that of β-Iminazolylethylamine on the other, on the isolated plain muscle of different organs. Acetyl-choline, as we have seen, has an activity on the intestinal muscle of the rabbit of the same order as that of β-Iminazolylethylamine on the uterine muscle (cf. Dale and Laidlaw).[15] To the latter base, on the other hand, the rabbit's intestine responds but feebly, doses of the order even of 1 in

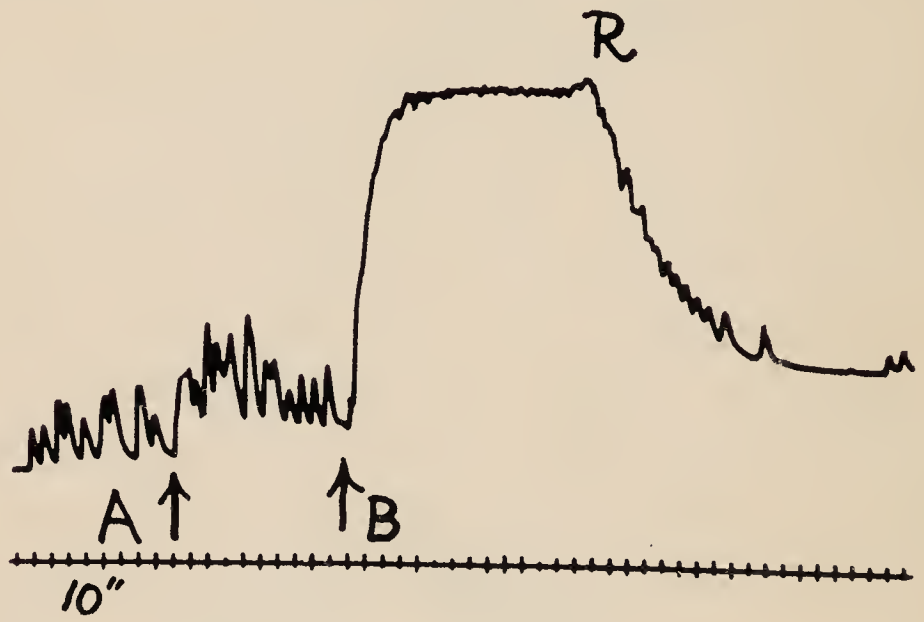


Fig. 15.

Lower end of cat's oesophagus in 50 cc. Tyrode's solution. At A 0.1 mgm. β-Iminazolylethylamine, at B 0.01 mgm. Acetyl-choline.

50,000 failing to produce maximal tonus. Other parts of the intestine—e.g. the rabbit's rectum—and the plain-muscular portion of the oesophagus show a similar high sensitiveness to the action of the choline-esters and relatively low sensitiveness to β-Iminazolylethylamine. Figure 15 shows a comparison of the effects on the lower part of the cat's oesophagus treated as an isolated organ.

The uterus. On the uterine muscle, on the other hand, the reverse relation holds, the action of β-Iminazolylethylamine being very much more powerful than that of the choline deriva-

[15] Dale and Laidlaw: Journ. of Physiol., xli, 318, 1910.

tives. The contrast is well-marked in the case of the guinea-pig's uterus, and still better in that of the cat's. It was shown by Guggenheim[16] that the uterine muscle of the non-pregnant rat was exceptional in being inhibited by β-Iminazolylethylamine. I have repeatedly confirmed this curious anomaly; this being the only known case in which β-Iminazolylethylamine has an action other than augmentor on the activity of isolated mammalian plain muscle. It was of interest, therefore, to observe

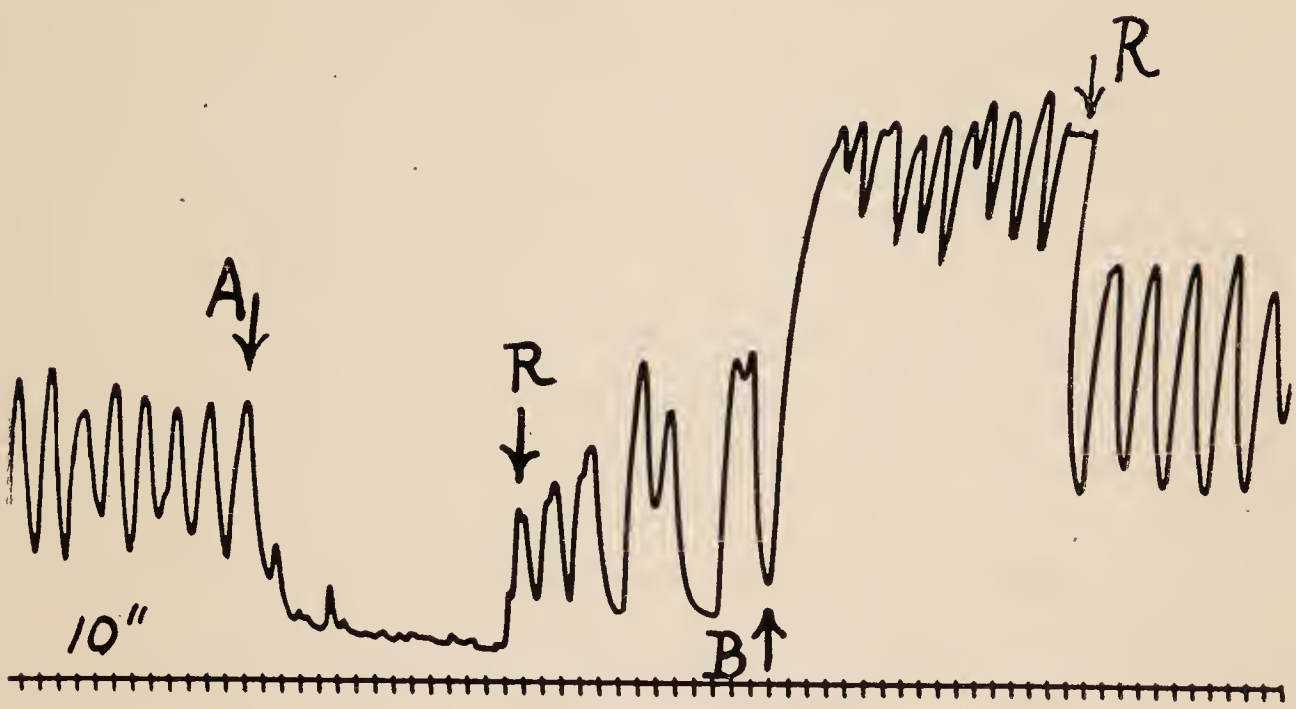


FIG. 16

Uterine horn of virgin rat in 50 cc. Tyrode's solution. At A 0.5 mgm. β1., at B 0.5 mgm. Acetyl-choline.

that the choline esters had their usual, rather weak stimulant action on the uterine muscle of this, as of other species (fig. 16).

The urinary bladder. On the urinary bladder all four choline derivatives have a stimulant action, that of acetyl-choline being again much less powerful and persistent than that of the other three when equal doses are given intravenously. Probably its inferiority may again be attributed to its smaller stability.

The eye. The action of these choline-derivatives on the plain muscle of the eye, and of the pupil in particular, has a somewhat special interest. One of the most characteristic actions of natural muscarine is the intense myosis which it produces in the

[16] Guggenheim: Therapeut. Monatsh., xxvi, Nov. 1912.

mammal. The pupil of the cat is reduced, under its action, to a narrow line, the edges coming apparently into complete apposition. The failure of "synthetic muscarine" to produce this intense myosis was one of the points emphasized by Böhm[17] as showing its difference from the natural base. I find that neither the acetic nor the nitrous ester ("synthetic muscarine") produces more than a trace of brief myosis when injected intravenously into the anaesthetized cat in a dose of 1 mgm. or more. We shall see that the effect is similarly deficient when larger doses of these esters are given hypodermically. On the other hand the nitric ester and the ethyl ester both produce the characteristic muscarine-myosis, the effect in either case being of maximal intensity and long persistence with 1 mgm. doses, given intravenously to the cat under ether. The effect is a purely peripheral one, being undiminished by removal of the ciliary ganglion. I have not yet tried it after degeneration of the post-ganglionic fibres. The action is abolished by a small dose of atropine.

Meyer[18] pointed out that the relation between the myotic efficiencies of the natural and synthetic muscarines, as seen in the mammal, was reversed in the case of the bird. He found that synthetic muscarine caused maximal myosis when instilled into the bird's eye in 1 per cent solution, while natural muscarine had no such effect. In the light of the facts that the bird's sphincter iridis is composed of striated muscle, and that the action of the third nerve thereon is paralyzed by curare and not by atropine, this observation would seem to fall into line with those recorded in other sections of this paper, pointing to the possession by choline nitrous ester (snythetic muscarine) of a nicotine-like action, which is wanting in the natural base. This interpretation, however, would lead to the anticipation that probably the nitric ester, and certainly the ethyl ether would have a weaker myotic effect, in the bird, than the nitrous ester. This anticipation is not fulfilled by the results of experiment.

[17] Böhm: loc. cit.

[18] Meyer: See Nothnagel, Arch. d. Pharm., ccxxxii, p. 286, 1894.

In the fowl I could not obtain a myotic effect by application of 1 or 2 per cent solutions of any of these substances to the eye. In the pigeon, however, well-marked effects were observed, though there was considerable individual variation. Instillation of a 1 per cent solution of the nitrous ester produced in some pigeons a strong myosis, in accordance with Meyer's description for synthetic muscarine. In others such a solution had little effect, and it was necessary to use a 2 per cent solution to obtain a strongly marked myosis. On the other hand, 1 per cent solutions of the nitric ester and ethyl ether were always powerfully myotic in the pigeon, and, in cases where direct comparison was made on the two eyes of one individual, a 1 per cent solution of either of these derivatives was approximately equivalent in action to a 2 per cent solution of the nitrous ester. In no case was the result affected by instillation of atropine.

The bronchioles. On the musculature of the bronchioles all four derivatives have the constrictor action familiar in the case of muscarine. In the case of acetyl-choline the effect is again a rapidly evanescent one, being far more persistent and powerful in the case of the other three, the ethyl-ether giving apparently the greatest intensity and persistence of effect with equal doses.

The retractor penis (*dog*). Hitherto the only involuntary muscle we have considered which receives an inhibitor nerve-supply, from the cranial or sacral division of the involuntary nervous system, is that of the heart. In this instance the inhibitor effect of the vagus is reproduced, as we have seen, by the choline derivatives. The plain muscle receiving a purely inhibitor supply from these divisions of the system includes that of certain arterioles, the urethral sphincter and the retractor penis. We shall deal in another section with the arterial dilatation which the choline derivatives produce in the genital area. As a separate tract of plain muscle the retractor penis offers unique advantages for recording a motor or inhibitor action, when such exists. I experimented on two dogs, under morphia and A. C. E. mixture. The retractor penis was dissected clear for about 2 cm. at its distal end and attached by a thread passing over a system of pulleys to a lever, which was drawn down against the tension of a

spring by contraction, and raised by the spring with relaxation of the muscle. When the experiment was finished the dog was killed, the retractor removed and suspended in Ringer's solution and its response again tested as an isolated organ. In the first experiment the action of acetyl-choline only was tested, the other derivatives not having, at that time, been brought into the investigation. In this experiment each injection of acetyl-choline produced a distinct relaxation of the muscle *in situ*. On the isolated muscle, however, I could obtain no definite result with this ester. This suggested to me that the relaxation recorded with the muscle in its natural relations might be due to the fall of blood-pressure which the ester produced. In a later experiment I tested this point, and found, indeed, that stimulation of the vagus was accompanied by a relaxation of the retractor not unlike that produced by injection of acetyl-choline. In the same experiment the nitric ester produced a small increase of tone in the muscle, both when injected intravenously with the muscle *in situ*, and when added to the bath containing the isolated muscle. The action was a very weak one as compared with that of the same esters on intestinal muscle, or with that of β-Iminazolyl-ethylamine on the retractor penis. The latter muscle seems, therefore, to be analogous to that of the uterus in its response to these bases, which seem to have a weak motor action on plain muscle as such, apart from its innervation.

Action on gland cells

All four derivatives cause a flow of saliva, tears and pancreatic juice. The effect on the pancreatic flow is a comparatively small one, and like all the other peripheral effects of these substances, readily abolished by atropine. The effect on the salivary flow gives the best opportunity of comparing the action of the different bases on gland cells. As in the case of other effects produced by injecting these substances intravenously, the effect of acetyl-choline is weak and evanescent as compared with those of the other three. The salivary flow, in response to an injection of a dose of 0.5 mgm. or so of this ester, starts after a brief interval,

attains for a short period a considerable rapidity, and then slows to complete arrest so suddenly as to suggest blocking of the duct, until it is found that a second, similar injection produces an exactly similar effect.

The profuse and long continued flow of saliva produced by the nitrous ester is well known from the observations of previous observers, who have regarded it as synthetic muscarine. A comparison between its effect and that of acetyl-choline is afforded by the following extract from an experimental record:

Experiment. Cat, ether. Salivary flow recorded from Wharton's duct. The figures indicate the movement of the meniscus in millimeters, along a horizontal tube attached to a scale, in 10 second periods. Injections by femoral vein. Chorda tympani and cervical sympathetic cut.

1. Effect of 0.1 mgm. of acetyl-choline. 0, 0, 0, Injection 6, 27, 6, 0, 0, 0. Total 39

2. Effect of 0.1 mgm. of choline nitrous ester 0, 0, 0, injection 5, 54, 34, 25, 21, 21, 22, 26, 31, 31, 36, 36, 35, 35, 30, 30, 30, 24, 22, 22, 19, 16, 14, 11, 13, 9, 10, 8, 7, 7, 7, 6, 6, 6, 4, 7, 5, 5, 3, 4, 3, 5, 2, 2, 2, etc. Total 825.

It is difficult to make a quantitative comparison between the sialagogue effects of the nitrous and nitric esters and the ethyl ether, the effect produced by even a small dose of any one of them being so prolonged that the effect of fatigue with successive doses can hardly be excluded, while it is difficult to assure constancy of other conditions over the long period required. So far as a comparison of results obtained in different experiments is any guide, it would seem that there is little difference between the actions of the nitrous and nitric esters in this direction, and that the effect of the ethyl-ether is the most persistent of all. In an experiment comparing its action with that of the much less active methyl-ether, from the record of which the following extract is taken, 0.5 mgm. of the ethyl ether produced a secretion of astonishing persistence, which showed no sign of becoming slower when the experiment was abandoned.

Experiment. Cat $2\frac{1}{2}$ kilos. Chloroform, then ether throughout; tracheotomy. Cannula in right Wharton's duct, connected to narrow tube on millimeter scale. Cannulae in left carotid artery, to record blood-pressure, and right femoral vein, for injections. Figures represent movement of salivary column along the tube in millimeters per interval of 10 seconds.

1. Effect of 1 mgm. of methyl-ether. 0, 0, 0, injection 14, 28, 22, 22, 20, 23, 20, 23, 21, 19, 21, 18, 18, 19, 15, 16, 15, 12, 13, 10, 11, 11, 10, 10, 8, 8, 5, 2, 3, 2, 2, 2, 1, 2, 1, 1, 1, 1, 0, 1, 0, 0, stops.

2. Effect of 0.5 mgm. of ethyl-ether. 0, 0, 0, injection 19, 21, 16, 11, 13, 12, 13, 13, 11, 12, 14, 13, 13, 12, 12, 11, 16, 14, 15, 14, 15, 13, 14, 14, 15, 12, 14, 13, 13, 13, 12, 13, 13, 13, 12, 14, 14, 15, 11, 14, 14, 14, 13, 14, 14, 14, 13, 12, 13, 14, 12, 13, 13, 14, 12, 14, 10, 7, 7, 9, 11, 10, 13, 11, 12, 11, 12, 11, 12, 11, 11, 12, 12, 10, 10, 10, 10, 11, 12, 12, 11, 12, 12, 13, 12, 11, 14.

Since the salivary flow showed no sign of becoming slower 15 minutes after the injection, the experiment was abandoned, and the cat killed.

The action of any of these derivatives on the salivary flow, in doses up to 1 mgm., is immediately annulled by a small dose of atropine. Ergotoxine, on the other hand, in doses sufficient to abolish entirely the secretomotor effect of adrenine, leaves the action of the choline derivatives hardly, if at all, affected.

All four substances produce a secretion of sweat from the hairless pads of the cat's foot, that caused by the ethyl-ether being apparently the most profuse and persistent. When acetylcholine is injected intravenously into the anaesthetized cat the effect may be so small and evanescent as to be barely perceptible. The action is readily demonstrated, however, by giving a large dose of the ester hypodermically into the unanaesthetized animal, or, still better, by injecting a minute droplet of 0.1 per cent solution directly into the pad of the foot. When the local anaemia, immediately resulting from the pressure of the injection, has passed off, the pad is seen to be flushed by vaso-dilatation, while beads of sweat exude from the pores and soon coalesce, so that the whole pad becomes pink and wet, while its neighbors remain relatively pale and dry.

The effect of hypodermic injections

In the preceding sections the effects on involuntary muscle and gland cells, as seen in the anaesthetized or brainless animal, or in isolated organs, have been described. There remains for description the combination of these and certain other effects, as seen when the choline derivatives are injected under the skin of the otherwise normal animal.

Action on the cat. As might be anticipated acetyl-choline, the effect of which is so evanescent, when it is injected directly into the circulation, is relatively very weak in its action when injected hypodermically. 10 mgm. of the hydrochloride, dissolved in 1 cc. of saline, and then injected into a large cat, produced no perceptible effect. 20 mgm., however, caused well-marked symptoms in a smaller cat, weighing about 2 kilos. These were mostly attributable to the effects described separately in preceding sections. The heart-beat was slowed from 300 to 158 per minute; saliva poured from the mouth, there was plentiful secretion of tears, and the pads of the feet became moist with sweat; borborygmi gave evidence of vigorous intestinal movement, a little slime was passed from the anus, and weak retching movements were made, without actual vomiting; the respiration became labored. The pupil was unaffected. An additional feature of the action, not seen in the anaesthetized or pithed animal, was erection of the penis. A little urine was passed. No feature, indeed, was wanting to complete the picture of a general stimulant action on the extra-sympathetic sections of the involuntary nervous system, except constriction of the pupil; and the only effect present which does not come under this heading is the secretion of sweat. All these effects are prevented by a preliminary dose of atropine, after which acetyl-choline appears inert.

It was necessary, in order to complete its identification with "synthetic muscarine," to examine the effect on the unanaesthetized cat of the nitrous acid ester with and without a preliminary dose of atropine. In both cases the effect corresponded exactly with the description given by Böhm.[19] When 10 mgm. of

[19] Böhm: loc. cit.

the nitrous ester are injected hypodermically into a normal cat the effects described as resulting from injection of acetyl-choline appear in much more violent form. The animal salivates profusely and vomits and defaecates repeatedly and with violence. The heart is strongly inhibited, the pads of the feet covered with sweat, and the penis maximally erected. Respiratory changes are obscured by the persistent vomiting. After a few minutes general muscular tremors set in, beginning in the hind-quarters and spreading rapidly, and shortly afterwards the cat dies in, apparently, asphyxial convulsions. The heart beats slowly for some time after respiration has stopped. The pupil was very slightly narrowed at first and then dilated as asphyxia came on.

After a preliminary dose of atropine (5 mgm.) 10 mgm. of the nitrous ester cause none of the symptoms of involuntary nerve-stimulation. For about 10 minutes the animal is practically normal; then muscular tremors set in and the cat falls helpless on its side. The general muscular twitchings continue, and the respiration becomes very slow and irregular. In the only experiment of this kind which I made the respiration gradually improved again, and the cat after lying narcotized for several hours, recovered completely. These muscular tremors are very characteristic of the action of the nitrous ester, and the effect observed corresponds exactly with that given by Böhm for "synthetic muscarine," administered to the atropinized cat in this dose. With larger doses he observed death from a curare-like paralysis of respiration.

The nitric acid ester I have given to the unanaesthetized cat only after atropine. The dose was again 10 mgm. No muscular tremors were produced, and the only effect, apart from a slight prostration, was excessively rapid respiration, which passed off after about an hour. This same contrast between the two esters is seen when both are given intravenously, to the pithed and atropinized cat, in large doses (5 mgm.). The nitrous ester causes general muscular twitchings, while the nitric ester causes only a trace of such an effect. No such effect is seen in the case of the ethyl ether, on the pithed and atropinized cat. I have not studied its action on the unanaesthetized mammal.

Action on the frog. On the frog, which has received previously 0.1 mgm. atropine sulphate, but is otherwise normal, acetyl-choline, in doses up to 1 mgm. has no effect of any kind. The nitrous and nitric esters, on the other hand, have the characteristic effect of "synthetic muscarine" as described by Böhm[20] and confirmed by Honda.[21] This is commonly described as "curare-like," but has features suggesting rather the action of nicotine. When a frog of 30 grams, which has previously been injected with 0.1 mgm. atropine sulphate, is given a dose of 0.4 to 1 mgm. of either of these esters, the first effect noted is a slowing and irregularity of respiration and a stiffness of movement. The latter soon becomes more marked, the fore-limbs in particular becoming stiffly adducted, so that the animal progresses by crawling, with the abdomen raised high off the table. The adduction of the fore-limbs becomes more and more marked, until they are clasped across the chest, as in nicotine-poisoning. Meanwhile voluntary movements of the hind-limbs cease, and the frog lies motionless, with the fore-limbs rigidly clasped, the abdominal muscles contracted, and the hind-limbs paralyzed, but less rigid.

The ethyl-ether produced a very similar effect under similar conditions, but was less toxic than the nitrous and nitric esters. Thus, with either the nitrous or nitric ester, a frog receiving 0.2 mgm. was slightly, but definitely affected, another receiving 0.4 mgm. showed complete paralysis but recovered, while frogs receiving 0.6mgm. or more died; with similar frogs 1 mgm. of the ethyl ether did not always kill, while recovery from lower doses was regular. It is possible that with a longer series of frogs, the difference would appear less marked; but the ethyl-ether is certainly less toxic to the frog after atropine than the other two. It may be noted that the activities of these substances in producing the more characteristic "muscarine" action, in the absence of atropine, are in the reverse order.

In common with previous workers I find that natural muscarine (Amanita extract) in large doses has no effect on the atropinized frog.

[20] Böhm: loc. cit.
[21] Honda: loc. cit.

Action of certain allied derivatives

These are all weaker in action than those previously dealt with and, in most cases, the action on the etherized cat, without atropine, has alone been studied. Of the long series studied by Hunt and Taveau none, save acetyl-choline, has been reëxamined.

Choline esters. The following additional ones have been examined.

1. Formyl choline – $HO.N(CH_3)_3.CH_2.CH_2.O.OCH$

This resembles its homologue, acetyl-choline, in the type of its action. With medium doses (e.g. 0.1 mgm.) a brief, intense cardiac inhibition, succeeded by a fall of pressure with accelerated heart-beat is produced. It is far less active than acetyl-choline.

2. Lactyl choline –

$$HO.N(CH_3)_3.CH_2CH_2O.OC.CHCH_3$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad |$$
$$HO.N(CH_3)_3.CH_2CH_2O$$

This partakes of the nature of an ether and an ester, two molecules of choline being united to one lactic acid residue. It is not notably more active than choline itself.

3. Choline sulphuric acid ester –

$$N(CH_3)_3.CH_2.CH_2.O.SO_2.O$$

This ester, which has a betaine-like structure, is considerably more active than choline, but very weak in comparison with the nitrous ester, etc.

Choline ethers. In addition to the highly active ethyl ether, the methyl ether $HO.N(CH_3)_3CH_2CH_2.O.CH_3$, and the propyl ether, $HO.N(CH_3)_3.CH_2CH_2OC_3H_7$, were made and studied for comparison. The methyl ether has an action qualitatively very similar to that of the ethyl-ether, but much weaker (fig.17). It has not more than $\frac{1}{5}$ of the activity of its homologous neighbor. The propyl ether is very feebly active—little more so than choline itself. In this series, then, the activity of the ethyl ether forms a well-marked climax.

Dicholine ether,

$$HO.N(CH_3)_3.CH_2\,CH_2\,O.CH_2\,CH_2\,N(CH_3)\,OH,$$

was also tested, but proved not more active than choline.

Other derivatives. Derivatives in which the alcoholic hydroxyl of choline is replaced by a halogen atom, or an amino-group, have also been examined. They are:

Trimethyl-chlorethyl ammonium chloride $-Cl.N(CH_3)_3.CH_2CH_2Cl$.

Trimethyl-bromethyl ammonium bromide $-Br.N(CH_3)_3CH_2CH_2Br$.

Trimethyl aminoethyl ammonium hydroxide $-HO.N(CH_3)_3CH_2CH_2NH_2$.

These all show a marked advance on choline in activity. In the case of the chloro- and bromo-derivatives, a dose of 5 mgm.

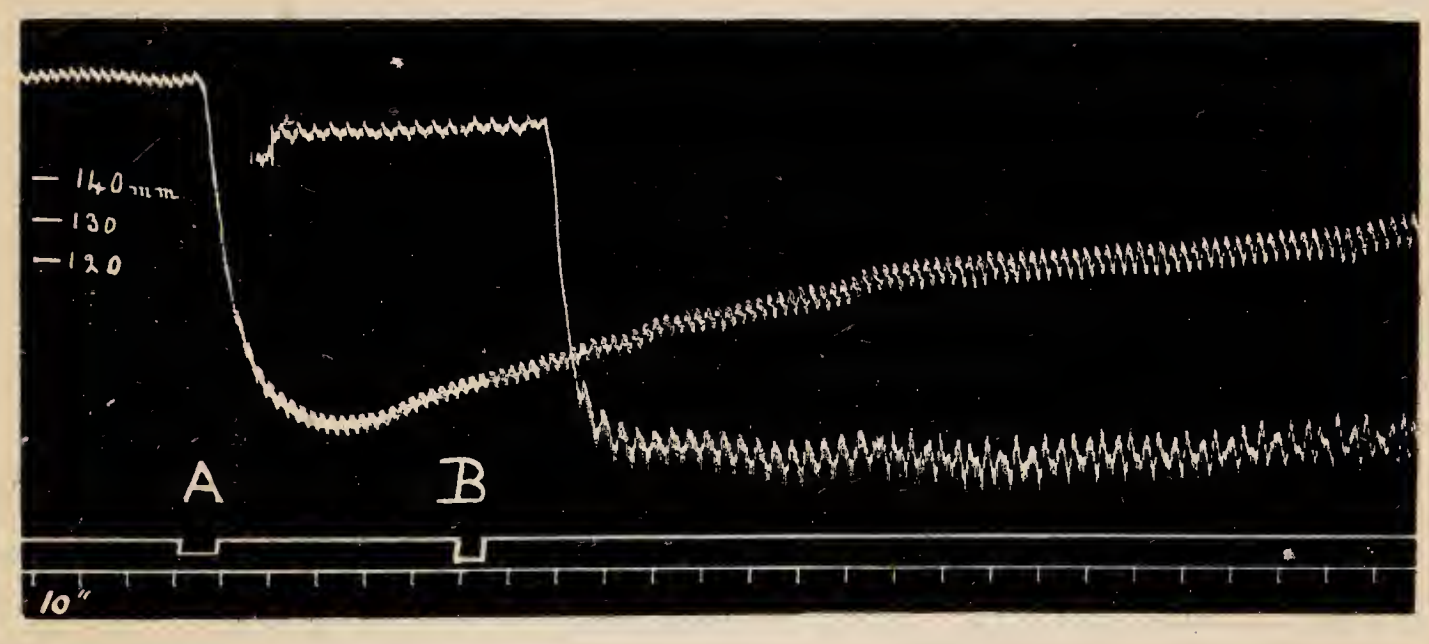


FIG. 17.

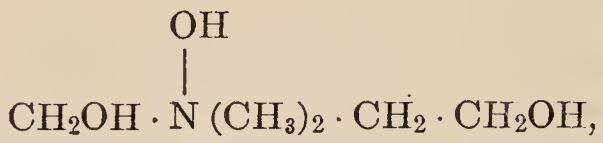
Cat: ether. Carotid blood-pressure. Intravenous injections. At A 1 mgm. choline methyl-ether, at B 0.5 mgm. choline ethyl-ether.

produces a muscarine-like effect comparable to that produced by 0.5 mgm. of the nitrous ester. The animo-base is even more active; 1 mgm. produces a well-marked muscarine-like slowing of the heart, with definite myosis. It has, apparently, about $\frac{1}{4}$ to $\frac{1}{2}$ of the activity of the nitric acid ester.

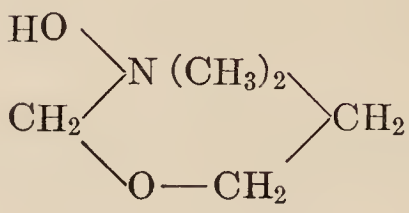
A derivative constructed by substituting a CN radicle for the alcoholic OH of choline showed activity complicated by toxicity of a different kind, which deprived it of interest for our immediate purpose.

An attempt to make a substance, corresponding in empirical

formula with the analysis for natural muscarine given by Harnack, and having the structure



resulted in ring formation, with the elimination of water.



This substance, dimethyl oxazolinium hydroxide, showed no activity of any kind.

DISCUSSION AND SUMMARY

The experiments described in the foregoing sections, starting with the identification of the muscarine-like substance in ergot as acetyl-choline, have not yet led, as was hoped, to the elucidation of the chemical structure of true muscarine. Some of the observations made in the course of the attempted synthetic identification are, however, not without theoretical interest.

In the first place, it has been shown that the substance obtained by the action of fuming nitric acid on choline, and identified by Ewins as the choline ester of nitrous acid, is in every respect physiologically identical with the "synthetic muscarine" prepared in this manner by Schmiedeberg and Harnack. It has been shown to possess most of the actions of true muscarine, with the characteristic exception of the intense myotic action, and to possess, in addition, an action of a different type, which may for brevity be called a "nicotine-action," and which was indicated by Böhm as complicating the action of "synthetic muscarine." Further investigation showed that these two distinct types of activity could be recognized in various choline derivatives and even in choline itself. Their main features are briefly as follows:

(1) "Muscarine" action—i.e., the action which true muscarine exhibits in its pure form, uncomplicated by the nicotine action. This has long been familiar in the case of muscarine itself, and I have described its production by a number of the choline derivatives. As numerous writers have pointed out, it may be summarized, with but small qualification, as a reproduction of the effects of stimulating nerves belonging to the cranial and sacral divisions of the involuntary (autonomic) system, so that, in the case of organs receiving a double and antagonistic nerve-supply, from this part of the system and from the true sympathetic, the muscarine effect is frequently antagonistic to that of true sympathetic nerves and adrenine. The constrictor effect of the third cranial nerve on the pupil; the powerful secretomotor action of the chorda tympani on the salivary glands; the inhibition of the heart, constriction of the bronchioles, and contraction of the muscular walls of the alimentary canal, produced by the vagus; the motor action of the pelvic nerve on the large intestine and urinary bladder, and its vasodilator action on the external genitalia; all these are reproduced faithfully by muscarine, and by several of the choline derivatives with which this paper deals, with an intensity exceeding that with which the same type of action is produced by substances having no chemical relation to this group, such as pilocarpine. The parallelism with the effects of cranio-sacral involuntary nerves is not, indeed, perfect. The sweat-glands, with their anatomically sympathetic nerve-supply, but ready response to muscarine and the choline derivatives, break the perfection of the correspondence, as they do, indeed, in the case of any such comparison between drug action and involuntary nerve-supply. In other directions, also, the parallelism is not strict. Thus the choline derivatives produce vasodilatation not only in areas supplied by cranio-sacral vasodilators, such as the chorda tympani and nervi erigentes, but, apparently, over the whole arterial system, and even in areas such as the limbs, whither the extension of cranial or sacral involuntary nerves is anatomically not credible. The motor effect on the plain muscle of other viscera, again, while particularly pronounced in organs where a motor cranio-sacral nerve-supply

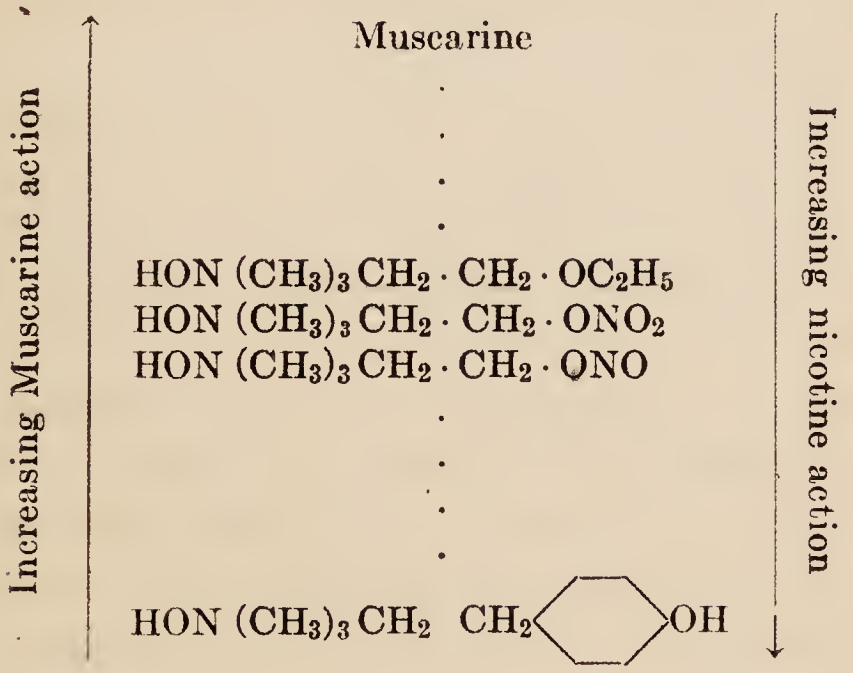
exists, as in the bronchioles and the alimentary canal, extends also in weaker form to an organ like the uterus, which has a purely sympathetic nerve supply, and that often purely inhibitor in function. On the whole, however, the correspondence is very striking, and little, if at all, less perfect than that between the actions of the true sympathetic system and of adrenine and its allies.

These "muscarine" effects are purely peripheral in their origin, unaffected by nicotine in large doses, but readily abolished by small doses of atropine. The evidence as to the exact position and nature of the structure primarily affected is incomplete. We have seen that removal of the ciliary ganglion has no immediate effect on the myotic action which some of these derivatives exhibit in such marked degree. Analogy with the action of pilocarpine,[22] in most respects so closely similar, suggests that post-ganglionic degeneration would leave the effect unchanged. On the other hand, Magnus[23] states that intestinal muscle only responds to muscarine when Auerbach's plexus is present, though the plexus-free preparation responds to pilocarpine. The point seems to need further investigation. In any case we may define the "muscarine" action as a peripheral action, broadly reproducing the effects of cranial and sacral involuntary nerves, and readily paralyzed by atropine.

(2) "Nicotine" action. The possession by quarternary ammonium bases of an action of the curare or nicotine type is one of the most familiar of pharmacological generalizations. The curare-like action of ammonium bases was pointed out by Crum-Brown and Fraser[24] and numerous later authors have added examples to the rule. The nicotine-like action of the quarternary bases producedby completely methylating adrenine and other "sympathomimetic" bases was described by Barger and myself.[25] It is not surprising, therefore, to find choline and its derivatives possessing an action which resembles that of nicotine, and, to

[22] Cf. Anderson: Journ. of Physiol., xxxiii, 414, 1905.
[23] Magnus: Pflügers' Arch., cviii, 1, 1905.
[24] Crum, Brown and Fraser: Journ. of Anat. and Physiol., i, 224, 1868.
[25] Barger and Dale: Journ. of Physiol., xli, 19, 1910.

some extent, that of curare. As described in preceding sections, this action is seen in uncomplicated form when the "muscarine" action is excluded by atropine, and can itself be suppressed by large doses of nicotine. The absence of the action in the case of muscarine may raise some doubt as to whether this substance be, indeed, a choline derivative and a quarternary ammonium base. There seems little doubt, however, as to its close chemical relationship with choline. In the case of the choline esters and ethers, we have seen evidence that, though the "muscarine" action and "nicotine" action are both increased by substituting the alcoholic OH group of choline in various ways, the intensities of the two types of action do not bear a constant relation to one another. Thus the ethyl ether has a more powerful muscarine action but a less powerful nicotine action than the nitrous ester, while the nitric ester is intermediate in both respects. We might, indeed, imagine a series of bases, the extreme terms of which were muscarine, on the one hand, and a completely methylated phenolic amine, such as hordenine methiodide, on the other; the ends of the series having, therefore, pure muscarine and pure nicotine action respectively. The choline derivatives, having both types of action, would be intermediate terms. Thus:



It would, of course, be impossible to include in such a series all the choline derivatives examined, still less all that might be made. We have evidence, in the case of the three ethers—methyl, ethyl,

and propyl—that both types of action may be increased very little, or both very much, without any obvious change in their relative intensities. The suggestion of such a series is merely intended, therefore, to emphasize the fact that choline and its derivatives have two distinct types of activity, which vary to some extent independently with modifications of the molecule, and which can each be seen in uncomplicated form in the action of a choline derivative, normally presenting both types of action, if one or the other type is suppressed by the previous administration of either atropine or nicotine in adequate dose. As I have already suggested, the presence of these two independent effects in the action of choline has led to the controversy as to whether the action of the pure base is depressor or pressor, since either type of action can be rendered predominant by suitable preparation of the animal.

In the consideration of any such series as that outlined above, acetyl-choline, probably with the similar but less active formyl-choline, occupies a peculiar position. Its "muscarine" action can be shown to be an extremely powerful one when the observation is made on isolated organs. When it is injected intravenously the action on most organs is cut short and weakened; the reason for this, as I have suggested, being its extreme liability to hydrolytic decomposition. This makes it impossible to form any accurate idea of its "nicotine" action, since this cannot be studied satisfactorily outside the body. The rise of blood-pressure which it produces after atropine shows that this action of choline is also intensified by acetylation, but no more definite statement can be made.

The action of the choline derivations on the pupil, in mammals and birds, also needs special mention. We have seen that the choline nitrous ester has very little effect on the mammalian pupil, this being a point emphasized by Böhm as distinguishing "synthetic" from natural muscarine. Meyer, on the other hand, showed that "synthetic" muscarine (i.e. choline nitrous ester) caused constriction of the bird's pupil, while the natural muscarine had not this action. Having regard to the fact that the bird's *sphincter iridis* is composed of striated muscle, this could

well be interpreted as a "nicotine" action, and it is in accord with this view that it is unmodified by atropine. In that case one would expect that the nitric ester and ethyl ether, with their intense muscarine-like action on the mammalian pupil, and, in the case of the ethyl ether, comparatively weak nicotine-like action, would be less efficient constrictors of the bird's pupil. The experimental comparison shows that they are both, in fact, more potent myotics, in this case also, than the nitrous ester.

It is clear, then, that the distinction between "muscarine" and "nicotine" activity cannot be made with absolute sharpness, when effects on different species are included in the comparison. Nor is there any evidence enabling us to regard one group of the molecule as responsible for the one type of action, and another for the other. One can merely conclude that there is some degree of biochemical similarity between the ganglion cells of the whole involuntary system, and the terminations of voluntary nerve-fibers in striated muscle, on the one hand, and the mechanism connected with the peripheral termination of cranio-sacral involuntary nerves on the other. We have a whole group of substances which affect both, and, in the case of the bird's sphincter iridis, a structure which in some respects forms a connecting link between the two types of biochemical affinity.

There does not seem to be the same relation between the affinities of ganglion cells and of the terminal mechanism connected with the true sympathetic system. By methylating completely the animo-group of a sympathomimetic amine one might expect that a base would be produced which would have a nicotine action and a sympathomimetic action. In no case has this been observed. In some cases, as shown by Barger and myself, a substance of purely nicotine-like action results (e.g. hordenine methiodide). On the other hand from sympathomimetic bases of the simple aliphatic series, such as isoamylamine and hexylamine, quarternary ammonium bases are produced which, as was shown long ago by Schmiedeberg and Harnack,[26] and by Jordan,[27] combine a muscarine and nicotine action, like the choline

[26] Schmiedeberg and Harnack: Arch. f. exp. Path. u. Pharm., vi, 101, 1877.
[27] Jordan: Arch. f. exp. Path. u. Pharm., viii, 15, 1878.

derivatives here studied. A similar combination of muscarine- and "curare"-action was observed by Jacobi and Hagenberg in tetramethyl- and tetraethyl-ammonium-triiodides.

A curious result of the investigation is the fact that the substitution of the alcoholic OH group of choline in almost any way leads to a greater or less, and frequently to a very great increase of activity, and that both the types of activity described above are discoverable in most cases of such intensified action. Nor are the substituting groups such as produce increase of activity, with little change of type, in most classes of compounds. Most esters and ethers, for example, are relatively inert substances physiologically; yet among the esters and ethers of this series we find some of the most potent of all known substances having an immediately toxic action.

The question of a possible physiological significance, in the resemblance between the action of choline esters and the effects of certain divisions of the involuntary nervous system, is one of great interest, but one for the discussion of which little evidence is available. Acetyl-choline is, of all the substances examined, the one whose action is most suggestive in this direction. The fact that its action surpasses even that of adrenine, both in intensity and evanescence, when considered in conjunction with the fact that each of these two bases reproduces those effects of involuntary nerves which are absent from the action of the other, so that the two actions are in many directions at once complementary and antagonistic, gives plenty of scope for speculation. On the other hand, there is no known depôt of choline derivatives, corresponding to the adrenine depôt in the adrenal medulla, nor, indeed, any evidence that a substance resembling acetyl-choline exists in the body at all. Reid Hunt found evidence of the existence of a substance in the supra-renal gland, which was not choline itself, but easily yielded that base in the process of extraction. If acetyl-choline, however, or any substance of comparable activity, existed in the supra-renal gland in quantities sufficient for chemical detection, its action would inevitably overpower that

[28] Jacobi and Hagenberg: Arch. f. exp. Path. u. Pharm., xlviii,, 48, 1902.
[29] Reid Hunt: Amer. Journ. of Physiol., iii, p. xviii, 1899, and v, p. vi, 1901.

of the adrenine in a gland extract. The possibility may, indeed, be admitted, of acetyl-choline, or some similarly active and unstable ester, arising in the body and being so rapidly hydrolysed by the tissues that its detection is impossible by known methods. Such a suggestion would acquire interest if methods for its experimental verification could be devised.

There remains for discussion the occurrence of acetyl-choline in ergot, and the possibility of its presence contributing to or interfering with the therapeutic action of the drug. Its occurrence is much more irregular than that of the other known active principles. Out of a large number of specimens of ergot, examined during the past ten years, I have only found some five or six, which contained it in such proportion, that its action was clearly recognisable in that of the extract. Even in those cases the quantity present was so small as to exclude any possibility of its producing any perceptible effect, if the extract were given by the mouth, or even hypodermically, in such doses as come within the range of therapeutic practice. The largest proportion of acetyl-choline which I have yet found in an ergot extract was 0.2 mgm. per cc., according to a physiological estimation. Such a quantity could not produce any noticeable effect, unless the extract were injected intravenously. In the latter case it might cause a brief, but alarming inhibition of the heart's action. In preparations from ergot intended for intravenous injection, the presence of acetyl-choline, in quantity sufficient to produce this action, is clearly undesirable. We have seen in previous sections that its action on uterine muscle is weak, when compared with that of iminazolylethylamine on this organ, or with its own effect on intestinal muscle. In no respect, therefore, is it to be regarded as a valuable constituent of ergot.

No information is available which throws any light on the mode of formation of acetyl-choline in ergot, or on its significance in the metabolic processes of the fungus. It seems not unlikely, now that its natural occurrence has been demonstrated, that it may be found in extracts from other fungi, and possibly from animal tissues.

The following are the chief conclusions reached in this paper:

1. In the action of choline, and, with varying degrees of intensification, in the action of certain ethers and esters of choline, two distinct types of action can be detected—a "muscarine" action, paralysed by atropine, and a "nicotine" action, paralysed by excess of nicotine.

2. Among choline derivatives showing these types of action with marked intensity are acetyl-choline, choline nitrous ester (which is identical with the so-called "synthetic muscarine" of Schmiedeberg and Harnack), choline nitric ester, and choline ethyl ether. Other analogous derivatives show these actions with less intensity.

3. When a number of quarternary ammonium bases is examined, these two types of action are found to vary to some extent independently; certain such bases have a pure nicotine action, and true muscarine, if (as is still doubtful) it is an ammonium base, represents a type in which the nicotine action is absent. The choline esters and ethers so far examined occupy an intermediate position.

4. Acetyl-choline occurs occasionally in ergot, but its instability renders it improbable that its occurrence has any therapeutic significance.

It is a pleasure to acknowledge debts of gratitude to Prof. W. Wiechowski and Dr. O. Rosenheim, who generously placed at our disposal supplies of dried Amanita muscaria and of extract therefrom.